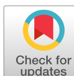

Histone H4 lysine 16 hypoacetylation is associated with defective DNA repair and premature senescence in Zmpste24-deficient mice

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Specific point mutations in lamin A gene have been shown to accelerate aging in humans and mice. Particularly, a de novo mutation at G608G position impairs lamin A processing to produce the mutant protein progerin, which causes the Hutchinson Gilford progeria syndrome. The premature aging phenotype of Hutchinson Gilford progeria syndrome is largely recapitulated in mice deficient for the lamin A-processing enzyme, Zmpste24. We have previously reported that Zmpste24 deficiency results in genomic instability and early cellular senescence due to the delayed recruitment of repair proteins to sites of DNA damage. Here, we further investigate the molecular mechanism underlying delayed DNA damage response and identify a histone acetylation defect in *Zmpste24*$^{-/-}$ mice. Specifically, histone H4 was hypoacetylated at a lysine 16 residue (H4K16), and this defect was attributed to the reduced association of a histone acetyltransferase, Mof, to the nuclear matrix. Given the reversible nature of epigenetic changes, rescue experiments performed either by Mof overexpression or by histone deacetylase inhibition promoted repair protein recruitment to DNA damage sites and substantially ameliorated aging-associated phenotypes, both in vitro and in vivo. The life span of *Zmpste24*$^{-/-}$ mice was also extended with the supplementation of a histone deacetylase inhibitor, sodium butyrate, to drinking water. Consistent with recent data showing age-dependent buildup of unprocessable lamin A in physiological aging, aged wild-type mice also showed hypoacetylation of H4K16. The above results shed light on how chromatin modifications regulate the DNA damage response and suggest that the reversal of epigenetic marks could make an attractive therapeutic target against laminopathy-based progeroid pathologies.

Eukaryotic cells are equipped with a surveillance machinery to orchestrate the rapid detection and repair of DNA damage. When DNA damage occurs, chromatin surrounding the double-strand breaks (DSBs) is altered and histones are modified to facilitate access for repair proteins (1). As a rapid response to DSB induction, the histone H2A variant, H2AX, is phosphorylated at Ser139 (γ-H2AX), which in turn interacts with MDC1, a DSB repair mediator, to facilitate the further recruitment of DNA repair proteins, such as 53BP1 and BRCA1 (2–4). Interestingly, γ-H2AX accumulation has been documented both in human senescent cells and in the fibroblasts of aged mice and primates (5–8). It has been proposed that these age-associated γ-H2AX foci contain nonrepairable DSBs and may have a role in initiating aging, especially because DSBs are very toxic and are one of the most lethal forms of DNA damage. Direct evidence for nonrepairable DNA damage as an inducer of premature aging has been obtained from mouse models that lack DNA repair proteins, such as ATM, Ku70, Ku80, DNA ligase IV, and Ercc1, as well as from humans with premature aging syndromes (9, 10). Together, these studies support the idea that the inability to recruit repair proteins to sites of DNA lesions leads to the accumulation of irreparable DNA damage, which predisposes cells to premature senescence, growth retardation, and accelerated aging (11).

Zinc metallopeptidase, STE24 homolog (Zmpste24) is a metalloproteinase responsible for the posttranslational processing and cleavage of prelamin A into mature lamin A. The *Zmpste24*$^{-/-}$ mice exhibit premature aging features characterized by hair loss, growth retardation, osteoporosis, and premature mortality (12). These progeroid features are also manifested in Hutchinson Gilford progeria syndrome (HGPS) patients, a syndrome that arises due to a de novo lamin A gene (*LMNA*) point mutation giving rise to a truncated protein, progerin (13). In our previous work, a relationship between genomic instability and premature aging was established (14). We showed that *Zmpste24*$^{-/-}$ mouse embryonic fibroblasts (MEFs) and HGPS cells in culture accumulated DNA damage and chromosome aberrations. *Zmpste24*$^{-/-}$ MEFs were highly sensitive to a wide variety of DNA-damaging agents and exhibited early cellular senescence in culture. In particular, upon DSB induction, a significant delay in the recruitment of 53BP1 and Rad51 was noted in Zmpste24-null cells and HGPS fibroblasts, a finding corroborated later by several other reports (15–18). Together, these studies have led to the idea that prelamin A/progerin accumulation interferes with the loading of DNA repair proteins to DSB sites and that, as a consequence, nonrepairable DNA damage remains and early cellular senescence occurs to cause premature aging.

The proper conformation of chromatin is essential for the recruitment of DNA repair proteins to DSBs. In response to DNA damage, chromatin structure is altered by enzymes that remodel chromatin or carry out the epigenetic modification of histones. Consequently, chromatin surrounding DSBs undergoes relaxation to generate a compartment capable of recruiting and retaining repair factors (19–21). We hypothesized that mutant lamin A might have a severe effect on chromatin organization due to aberrant epigenetic modification of histones. Recent reports have shown that histone H4 acetylation at lysine 16 (H4K16) plays an important role in DNA damage response and DSB repair (22, 23). Specifically, H4K16 acetylation neutralizes the positive charge on the H4 tail, which then poses a structural constraint on the formation of higher-order chromatin and forces the chromatin to stay in a more open configuration.

Here, we describe impaired histone H4 acetylation in the Zmpste24-deficient cells. Our data indicate that reduced H4K16 acetylation contributes to the delayed recruitment of DNA repair proteins in *Zmpste24*$^{-/-}$ MEFs. Furthermore, pharmacological modulation of histone acetylation was found to delay premature cellular senescence and aging. Together with similar epigenetic patterns observed in wild-type mice during normal physiological aging, these findings not only provide a paradigm

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for the role of epigenetic signaling in DNA damage repair, but also extend the mechanistic link between histone modifications, genomic instability, and aging.

Results

Reduced H4K16 Acetylation in *Zmpste24*-Deficient MEFs. To evaluate the association between epigenetic events and aging, we compared the histone modification patterns between wild-type and *Zmpste24*$^{-/-}$ mutant cells. Under identical culture conditions, *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were negative for senescence-associated β-galactosidase (SA β-gal) staining at early passage (passage 3) (Fig. 1*A*, *Upper* panels). However, upon continuous passaging (passage 6), ∼60% of the *Zmpste24*$^{-/-}$ MEFs showed SA β-gal positivity and flattened morphology (Fig. 1*A*, *Lower* panels). To study whether early senescence in *Zmpste24*$^{-/-}$ MEFs was caused by altered chromatin modifications, histone modification at specific sites was examined in early passage MEFs. Western blotting analyses demonstrated that global H4 acetylation was down-regulated in *Zmpste24*$^{-/-}$ MEFs (Fig. 1*B*). Acetylation at particular H4 lysine residues was further examined. Whereas H4K8 and H4K12 acetylations were slightly lower, the acetylation at K16 was reduced by about 50% in the *Zmpste24*$^{-/-}$ mutant (Fig. 1 *C* and *D*). Consistently, immunofluorescence staining for H4K16 acetylation revealed a 50% reduction in *Zmpste24*$^{-/-}$ MEFs compared with the wild-type control (Fig. 1*E*). Hypoacetylations on H4 and H4K16 were also observed in the liver of 1-mo-old *Zmpste24*$^{-/-}$ mice, a time point at which the mice had not yet developed any signs of premature aging (Fig. 1*F*). Then, to ensure that the down-regulation of H4 acetylation was not a reflection of global aberrations in epigenetic modifications, other modification sites on histone H3 and H4 were analyzed. No significant alteration could be detected in the overall H3 acetylation, H3K79 di-methylation, or H4K20 di-methylation (Fig. S1*A*). The relationship between prelamin A and H4 acetylation was further investigated using HEK293 cells that ectopically expressed unprocessable prelamin A (C-terminal Ser-Ile-Met replaced with Phe-Phe-Met). There was a marked reduction of H4 and H4K16 acetylation in HEK293 cells upon transfection with unprocessable prelamin A (Fig. S1*B*). Thus, these results suggest that prelamin A accumulation impairs H4K16 acetylation.

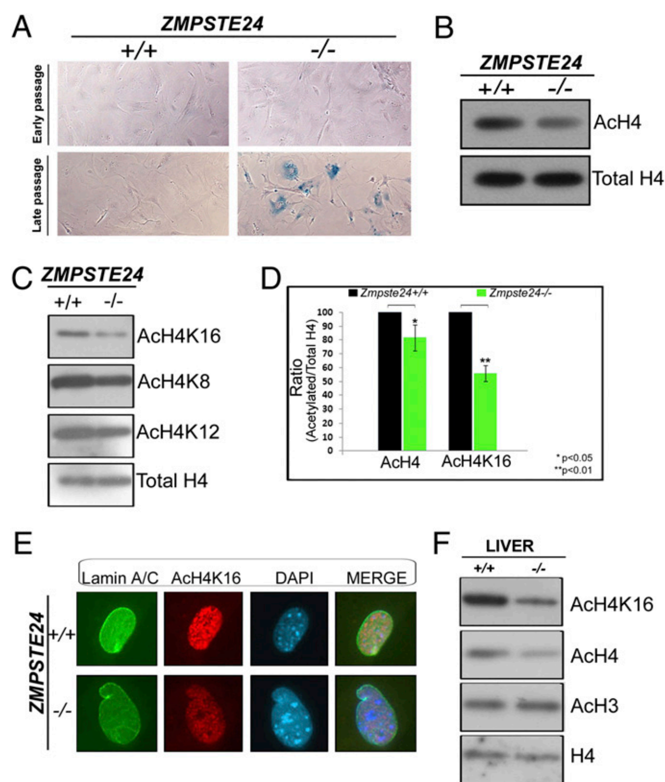

Fig. 1. Reduced H4K16 acetylation in *Zmpste24*$^{-/-}$ cells. (*A*) *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs at early passage (P3) (*Upper* panels) or late passage (P6) (*Lower* panels) were cultured for 2 d. SA β-gal activity was determined. (*B* and *C*) Histones were extracted from *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs (P3) and analyzed by Western blotting using the antibodies indicated. (*D*) Graph shows normalized densitometric values of acetyl H4 and acetyl H4K16 against total H4 for three independently derived lines of MEFs in separate experiments. $^{*}P < 0.05$ and $^{**}P < 0.01$ (Student's *t* test) when *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were compared. (*E*) Immunofluorescence staining of *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs with lamin A/C (green) and anti-acetyl H4K16 (red) antibodies. (*F*) Histones were extracted from the liver of 1-mo-old mice and analyzed by Western blotting using the antibodies indicated.

Prelamin A Accumulation Decreased Mof Association on the Nuclear Matrix. H4K16 acetylation in mammalian cells is catalyzed mainly by the histone acetyltransferase Mof (24, 25). Mof is important for the maintenance of genome integrity, and the depletion of Mof delays γ-H2AX foci formation and DNA damage response (22, 23). Because lamin A is an essential structural component of the nuclear matrix and because the nuclear matrix contains a network of proteins that facilitate chromatin organization and transcriptional regulation, we investigated whether Mof is one of the chromatin modifiers present at the nuclear scaffold. The localization of Mof was determined by a well-established immunocytological approach, in which the soluble proteins and DNA are removed by pre-extracting cells with a hypertonic buffer, detergent, and DNase sequentially, followed by immunofluorescence staining and confocal imaging of the residual nucleoskeleton (26). Compared with the negative control processed in parallel, the loss of DAPI staining in the isolated nuclear matrix indicated the complete digestion of DNA by DNase treatment. Immunofluorescence results revealed expression of Mof on the nuclear matrix, and almost 98% of Mof signal colocalized with that of lamin A in wild-type MEFs (Fig. 2 *A* and *B*). The association between Mof and lamin A was further examined in HEK293 cells that were stably expressing FLAG-tagged lamin A with transient ectopic expression of myc-tagged Mof. Lamin A coimmunoprecipitated with myc, and Mof was detected in flag precipitate (Fig. S2*A*).

To determine whether the accumulation of unprocessed prelamin A affects Mof localization in the cell, a subcellular fractionation assay was performed (Fig. S2*B*). Proteins from various subcellular compartments were separated into different fractions and immunoblotted with antibodies directed against ERK1/2, Mcm3 or histones, and lamin A/C as controls to confirm the purity of the cytosolic, chromatin-bound, and nuclear matrix fractions, respectively. Regardless of whether cells were exposed to DNA damage, total Mof levels did not differ between *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs (Fig. S2*C*). On the other hand, when Mof levels were compared after subcellular fractionation, the amount of nuclear matrix-associated (P2′ fraction) Mof was significantly lower in the *Zmpste24*$^{-/-}$ MEFs (Fig. 2*C*). Results obtained from HEK293 cells were similar, where a marked reduction of Mof on the nuclear matrix was detected upon prelamin A overexpression (Fig. S2*D*).

To further understand why less Mof was retained in the nuclear matrix in prelamin A-expressing cells, the binding of Mof to lamin A and prelamin A was compared. Equivalent amounts of FLAG-tagged lamin A and prelamin A were transiently expressed in HEK293 cells in conjunction with myc-tagged Mof. To ensure the complete solubilization of nuclear matrix-targeted proteins, lysates were prepared in the presence of high salt concentrations. Immunoprecipitation was performed with anti-FLAG conjugated beads, and immunoblots were probed with anti-myc antibody. Interestingly, the association of Mof to lamin A was several folds higher to lamin A compared with the binding of Mof to prelamin A (compare lanes 5 and 6, Fig. 2*D*). Taken together, the above biochemical data suggest that the accumulation of prelamin A interferes with the retention of Mof in the nuclear matrix and that Mof is mislocalized in prelamin A-expressing cells.

Mof Is Essential for 53BP1 Foci Formation at DNA Damage Sites. To examine the functional importance of Mof and H4K16 acetylation in the control of DNA damage repair and cellular senescence, we (*i*) cloned the cDNA fragment encoding for Mof into a

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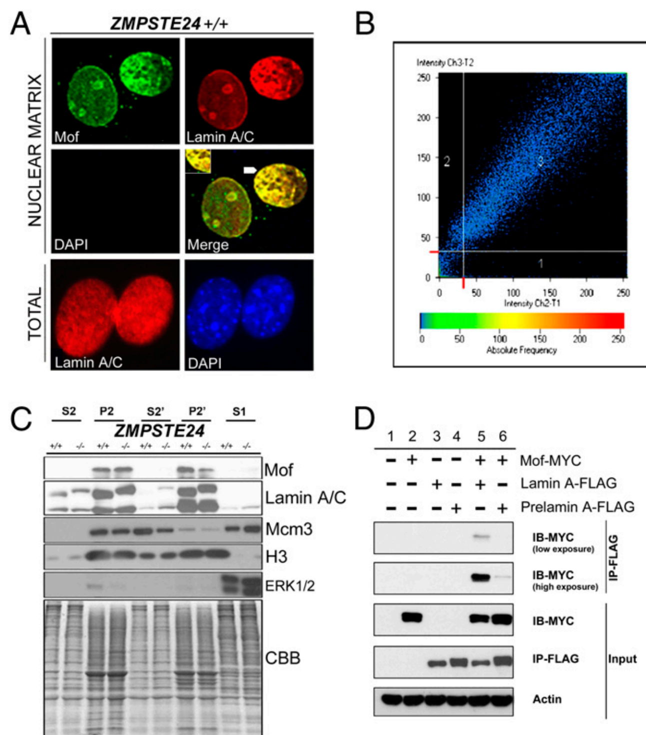

Fig. 2. Reduced nuclear matrix association of Mof in *Zmpste24*$^{-/-}$ MEFs. (*A* and *B*) Colocalization of Mof and lamin A in nuclear matrix. (*A*) Nuclear matrices were prepared from wild-type MEFs and stained with anti-Mof and anti-lamin A/C antibodies. Staining with DAPI indicated efficient digestion of DNA in the isolated nuclear matrix. Control cells (labeled "TOTAL") were processed directly without pre-extraction and stained with anti-lamin A/C antibody and DAPI. (*B*) Histogram:immunofluorescence signals collected from 488 channels (lamin A/C) and 594 channels (Mof) were overlaid to depict colocalization. (*C*) *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were subjected to subcellular fractionation to yield nucleoplasmic (S2), nuclear matrix + chromatin (P2), soluble chromatin + nucleoplasmic (S2′), nuclear matrix (P2′), and cytosolic (S1) fractions. Equal amounts of protein (20 μg) were resolved and Western-blotted with indicated antibodies. Coomassie brilliant blue (CBB) staining of the gel was performed to ensure equal loading of the fractions. (*D*) HEK293 cells were transiently transfected with Mof-myc together with FLAG-tagged lamin A or prelamin A for 48 h. As controls, cells were transfected with empty vector, FLAG-tagged lamin A, prelamin A or Mof-myc alone. Immunoprecipitation was performed with FLAG antibody-conjugated agarose beads, and immunoblots were probed with indicated antibodies. Three independent experiments were performed and identical results were obtained.

mammalian expression vector for transfection into *Zmpste24*$^{-/-}$ MEF, (*ii*) knocked down expression of endogenous Mof by siRNA, and (*iii*) exposed MEFs to HDAC inhibitors to increase histone acetylation levels.

When the EGFP-tagged Mof was expressed in *Zmpste24*$^{-/-}$ MEFs, global H4 acetylation was significantly up-regulated (Fig. 3*A*). The efficiency of the DNA damage response was then examined by immunofluorescence for γ-irradiation–induced repair-associated 53BP1 focus formation. MEFs were irradiated with 5 Gy at 48 h posttransfection. Pan-nuclear 53BP1 protein redistributed into distinct foci in *Zmpste24*$^{-/-}$ MEFs transfected with EGFP-tagged Mof at 30 min following irradiation (Fig. 3 *B* and *C*). By 120 min, the number of foci was restored to basal levels in Mof-transfected *Zmpste24*$^{-/-}$ MEFs, indicating that most of the damaged DNA was repaired. In contrast, the induction of 53BP1 foci formation was significantly delayed in the *Zmpste24*$^{-/-}$ MEFs with EGFP transfection, and the maximum recruitment of 53BP1 foci was detected at 120 min after DNA damage. On the basis of the above data, we conclude that exogenous introduction of Mof significantly improved 53BP1 recruitment in *Zmpste24*$^{-/-}$ MEFs.

H4K16 Hypoacetylation Leads to Early Cellular Senescence. To further establish a correlation between H4K16 acetylation and cellular senescence, we investigated the effect of Mof depletion. In line with earlier studies that H4K16 acetylation is abolished in Mof-null embryos, there was a ∼90% reduction in H4K16 acetylation in MEFs upon Mof knockdown with siRNA (Fig. 4*A*). Concomitant with reduced acetylated H4K16, the knockdown of Mof significantly exacerbated the early cellular senescence phenotype of *Zmpst24*$^{-/-}$ MEFs (Fig. 4 *B* and *C*). To confirm that Mof depletion leads to senescence even in wild-type cells, we transfected late passage wild-type MEFs that had not yet fully manifested cellular senescence phenotypes with Mof siRNA; and upon Mof knockdown, the percentage of SA β-gal–positive cells increased from an average of 48% to 73% (Fig. 4 *B* and *C*). Thus, reduced H4K16 acetylation strongly correlated with an increase in cellular senescence.

Consistent with our model, a reverse situation was observed when MEFs were treated with HDAC inhibitors. Upon incubation with the HDAC inhibitor, sodium butyrate (NaB), H4K16 acetylation increased whereas the percentage of senescent cells

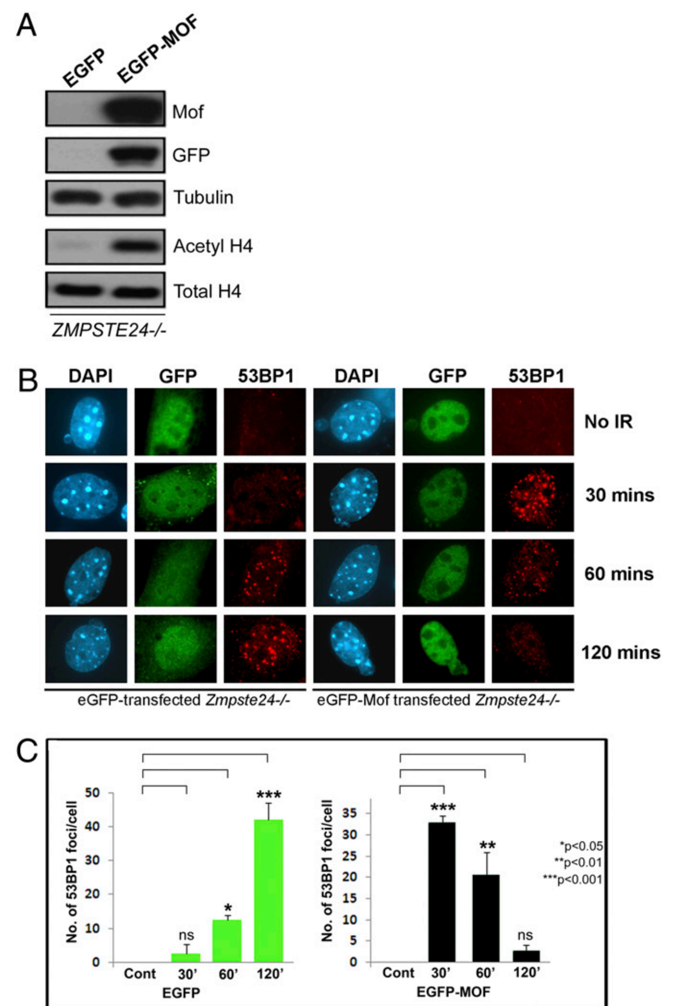

Fig. 3. Mof overexpression restores 53BP1 recruitment in *Zmpste24*$^{-/-}$ MEFs. (*A*) Total cell extracts from *Zmpste24*$^{-/-}$ MEFs transfected with EGFP or EGFP-Mof were Western-blotted with indicated antibodies. (*B* and *C*) EGFP or EGFP-Mof plasmids were transiently transfected into *Zmpste24*$^{-/-}$ MEFs for 48 h, followed by exposure to 5 Gy of γ-irradiation. Cells were fixed and processed for immunofluorescence at the indicated time points. Immunofluorescence images are represented in *B*. The number of 53BP1 foci per cell was counted and plotted in *C*. The controls and irradiated samples were compared using ANOVA. *$P < 0.05$; **$P < 0.01$; ***$P < 0.001$; "ns" indicates that the difference has not reached statistical significance.

was significantly reduced (Fig. 4 *B* and *C*). Because HDAC inhibitors may have off-target effects, we further validated the results using another HDAC inhibitor, trichostatin A (TSA). Both TSA and NaB promoted H4K16 acetylation and concurrently reduced senescence in a dose-dependent manner (Fig. 4 *D* and *E*). Similar results were obtained in prelamin A-transfected HEK293 cells subjected to NaB treatment. NaB promoted H4K16 acetylation (Fig. S3*A*), reduced cellular senescence (Fig. S3*B*), and improved cell survival after DNA damage (Fig. S3*C*).

Inhibition of HDAC Improves DNA Repair in *Zmpste24*$^{-/-}$ Cells. Given that histone acetylation is essential to promote an "open" chromatin conformation in DNA damage repair, we investigated whether chromatin relaxation caused by HDAC inhibition facilitated 53BP1 recruitment. The *Zmpste24*$^{-/-}$ MEFs were cultured and NaB was added 2 h before irradiation exposure to promote chromatin relaxation. At 30 min postirradiation, the number of 53BP1 foci was significantly higher in NaB-treated *Zmpste24*$^{-/-}$ MEFs compared with the untreated control (Fig. 5 *A* and *B*). Furthermore, the increased foci formation was comparable to that of the wild-type MEFs.

Despite certain limitations, there appears to be a close correlation between γ-H2AX foci quantification and other traditional procedures, such as pulsed field gel electrophoresis and comet assay, for examining the presence of nonrepairable DSBs (27). To examine if NaB pretreatment rescued the accumulation of irreparable DNA damage, MEFs were exposed to irradiation and processed for staining with antibody against γ-H2AX after 24 h. By 24 h, DSBs were mostly repaired in NaB-treated cells, as indicated by the significant reduction of γ-H2AX foci staining (Fig. 5 *C* and *D*). In summary, the induction of histone acetylation modified chromatin into an open confirmation, which facilitated 53BP1 recruitment to sites of DNA damage and reduced the accumulation of nonrepairable DNA damage in *Zmpste24*$^{-/-}$ cells.

Extension of Life Span Through HDAC Inhibition. The effect of HDAC inhibitors on accelerated aging was next extrapolated in vivo. NaB was added to drinking water and fed to parent mice. The treatment was continued throughout the life of the offspring and survival was monitored. Although the drug was toxic at 8 g/L, the life span of the *Zmpste24*$^{-/-}$ mice was significantly extended with a lower dose at 4 g/L. The survival curve comparison of the untreated and NaB-fed mice by Kaplan–Meir analysis revealed that the untreated group mice began to die at 16 wk of age, whereas the deaths in the NaB-treated group started at around 19 wk. At 22 wk of age, about 50% of the untreated *Zmpste24*$^{-/-}$ mice died, whereas 85% of NaB-fed *Zmpste24*$^{-/-}$ mice were still alive (Fig. 6 *A* and *B*). In addition, maximal life span also extended from 30 wk in the untreated group to 33 wk in the NaB-treated group. Bone X-ray scans showed that there was a statistically significant increase in bone density in the 5-mo-old NaB-fed *Zmpste24*$^{-/-}$ mice (Fig. 6 *C* and *D*). Because rapidly proliferating cells, such as the hematopoietic cells, accumulate DNA damage with aging (28), we quantified the extent of DNA damage accrual in the bone marrow. Concomitant with increased H4 acetylation, γ-H2AX levels were lower in circulating bone marrow cells in the NaB-fed mice (Fig. 6*E*). Furthermore, reduced SA β-gal staining was also observed in kidney sections of age-matched NaB-fed mice (Fig. 6*F*). In summary, the above results demonstrated that HDAC inhibitors attenuated premature aging pathologies, both in culture and in vivo.

Histone Hypoacetylation in Physiological Aging. Recently, lamin A-dependent nuclear defects have been shown to accumulate during

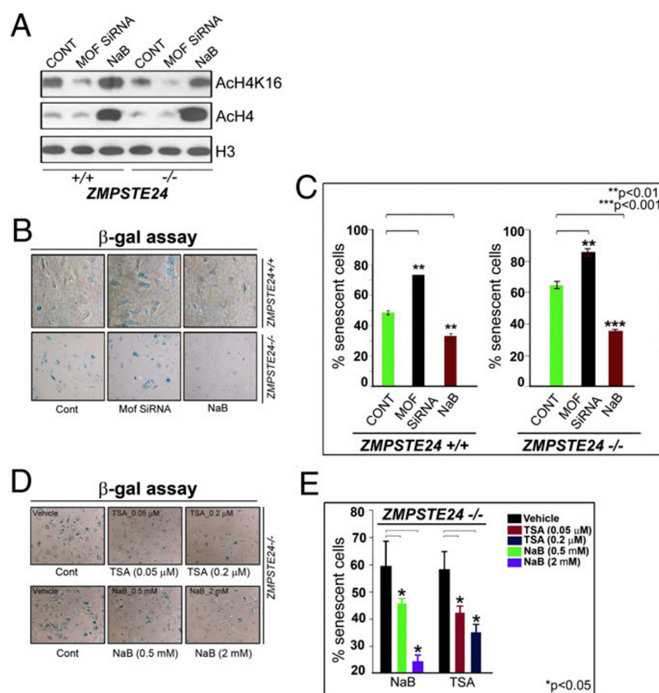

Fig. 4. Reduced H4K16 acetylation levels and early onset of cellular senescence. (*A*) *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were either subjected to Mof siRNA knockdown for 48 h or treated with NaB (2 mM) for 24 h. Total cell extracts were collected, and the effects of Mof siRNA and HDAC inhibition on histone acetylation were analyzed by Western blotting. (*B* and *C*) For the experiment described in *A*, senescent phenotype was studied using SA β-gal assay, and the percentage of senescent cells was quantified. $^{**}P < 0.01$ and $^{***}P < 0.001$ (Student's *t* test). (*D*) Treatment with NaB or TSA rescued early senescence in *Zmpste24*$^{-/-}$ MEFs, as determined by SA β-gal assay. (*E*) Quantitative data showing the percentage of senescent cells for the experiment described in *D*. $^{*}P < 0.05$ (Student's *t* test).

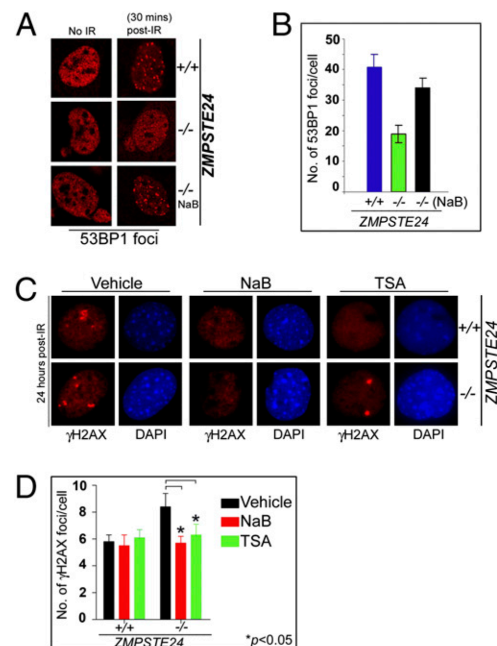

Fig. 5. HDAC inhibition rescues both delayed 53BP1 recruitment and defective DNA repair after γ-irradiation induced DNA damage. (*A*) *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were either mock-treated or treated with NaB (0.5 mM) for 2 h and exposed to 5 Gy of γ-irradiation, and immunofluorescence staining was performed using anti-53BP1 after 30 min. The number of 53BP1 foci per cell was quantified and shown in *B*. (*C*) Treatment with NaB (2 mM) or TSA (0.2 μM) improved DNA repair in *Zmpste24*$^{-/-}$ MEFs. Twenty-four hours postirradiation (5 Gy), *Zmpste24*$^{+/+}$ and *Zmpste24*$^{-/-}$ MEFs were fixed and the number of residual γ-H2AX foci per cell was counted. (*D*) Quantitative data showing the decrease of nonrepairable DNA damage in *Zmpste24*$^{-/-}$ MEFs treated with NaB (2 mM) or TSA (0.2 μM) for the experiment described in *C*. NaB- and TSA-treated samples were compared with untreated controls. $^{*}P < 0.05$ (Student's *t* test).

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normal human aging, and both prelamin A and progerin were reported as biomarkers in human skin and vascular aging (29–31). We set forth to examine whether normal aging exhibits similar change in histone acetylation. Our preliminary investigation with human tissue samples revealed a similar trend toward histone hypoacetylation with increasing age (Fig. S4). In addition, similar to prelamin A-expressing cells, global H4 acetylation and H4K16 acetylation decreased in an age-dependent manner in wild-type mice, suggesting the existence of commonalities in the molecular pathways participating in progeroid syndrome and physiological aging (Fig. S5 *A* and *B*). In conclusion, impairment in histone H4K16 acetylation appeared to be conserved across several aging cell types.

Discussion

Expanding on our previous work that describes genomic instability in *Zmpste24*$^{-/-}$ mice, this study provides further mechanistic insights into the importance of chromatin modifications in regulating DNA damage response, cellular senescence, and premature aging. Our results provide strong evidence that the accumulation of unprocessable prelamin A leads to the mis-positioning of Mof and to the hypoacetylation of its target, H4K16. On the basis of the reversible nature of histone modifications, we can say that increasing H4K16 acetylation by the ectopic expression of Mof or by HDAC inhibition promoted recruitment of repair proteins to DNA damage sites, reduced DNA damage accumulation, and significantly rescued early senescence of *Zmpste24*$^{-/-}$ MEFs in culture. The addition of NaB to drinking water also mitigated several progeroid phenotypes and extended the life span of *Zmpste24*$^{-/-}$ mice (model summarized in Fig. S5*C*).

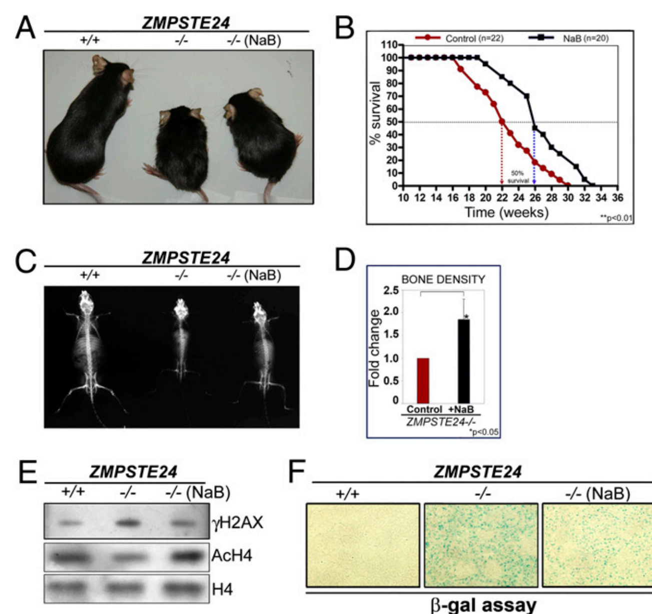

Fig. 6. HDAC inhibition mitigates progeroid phenotypes and extends life span of *Zmpste24*$^{-/-}$ mice. (*A*) Photograph of *Zmpste24*$^{+/+}$, *Zmpste24*$^{-/-}$, and NaB-fed *Zmpste24*$^{-/-}$ at 5 mo of age. (*B*) Survival and life span of the NaB-fed *Zmpste24*$^{-/-}$ mice (n = 20) were compared against the untreated controls (n = 22) using the Kaplan–Meir analysis and found to be statistically significant using the log-rank test (**$P < 0.01$). Fifty-percent survival was observed at 22 and 26 wk for *Zmpste24*$^{-/-}$ and NaB-fed *Zmpste24*$^{-/-}$ mice, respectively. (*C*) Five-month-old *Zmpste24*$^{+/+}$, *Zmpste24*$^{-/-}$, and NaB-fed *Zmpste24*$^{-/-}$ mice (n = 5) were scanned using X-ray for bone density analysis. (*D*) Bone densities were quantified from X-ray scans using ImageJ software (National Institutes of Health). Fold increase in the bone density of NaB-fed mice relative to the untreated *Zmpste24*$^{-/-}$ control is shown. *$P < 0.05$ (Student's t test). (*E*) Total cell extracts from bone marrow cells (5-mo-old mice) were resolved and Western-blotted with antibodies indicated. (*F*) Kidney was excised from 5-mo-old *Zmpste24*$^{+/+}$, *Zmpste24*$^{-/-}$, and NaB-fed *Zmpste24*$^{-/-}$ mice, cryosectioned, and stained using a SA β-gal assay kit. Blue cells indicate senescence.

Using immunofluorescence and biochemical fractionation methods, we show that MOF is anchored to the nuclear matrix and colocalizes with lamin A. Importantly, results from immunoprecipitation assays suggest that the association between Mof and lamin A is stronger than that between Mof and prelamin A. Because the function of DNA repair proteins and chromatin-remodeling enzymes is dependent on their proper positioning on the nuclear matrix (32), we hypothesize that the extra 18 amino acids at the carboxyl terminus of unprocessed prelamin A severely reduces the binding of Mof on the nuclear matrix and disrupts its histone acetylation functions. Intriguingly, apart from Mof, progerin expression has been shown to contribute to the mislocalization of ATR, SKIP, and XPA and to the degradation of NURD chromatin-remodeling complex subunits (16, 29, 33–35). The critical question that arises from these reports is why unprocessed lamin A expression elicits such pleiotropic outcomes. Investigation into whether these molecular defects are a direct consequence of nuclear structure disruption and reduced nuclear matrix binding will yield useful insights into the molecular mechanisms underlying premature aging.

Epigenetic alterations such as down-regulation of H3K9 trimethylation and H3K27 trimethylation and up-regulation of H4K20 trimethylation (36, 37) have been reported earlier in HGPS cells. Recently, histone H2B lysine 5 hypoacetylation was reported in *Zmpste24*-deficient mice (38). In addition to these reports, here we observed a reduction in H4K16 acetylation. Because H4K16 acetylation determines the ability of chromatin to fold into a higher-order structure, basal H4K16 acetylation creates a chromatin environment conducive for DNA damage recognition and DSB repair protein recruitment. Indeed, it has been shown recently that Mof depletion down-regulates H4K16 acetylation and delays IR-induced focus formation of 53BP1, Rad51, MDC1, γ-H2AX, and hSSB (22). Consistent with this scheme, modulation of basal H4K16 acetylation through Mof overexpression promoted 53BP1 recruitment to DNA damage sites in *Zmpste24*$^{-/-}$ MEFs.

Treatment of premature aging cells with HDAC inhibitors rescued their early senescence phenotype and extended the life span of Zmpste24-null mice in vivo. These results may appear surprising because HDAC inhibitors have been shown to be toxic and induce apoptosis in cell lines. However, the concentration of NaB used in our study was much lower, compared with the apoptosis-inducing concentrations. Also, our results are not unprecedented, given that HDAC inhibitors have been successfully used in several other age-related disease models as well. In particular, HDAC inhibition has been shown to significantly improve learning ability, delay age-dependent neurodegeneration, delay Alzheimer's disease progression in mouse models, accelerate age-associated osteogenesis, and increase life span of worms in a dietary restriction model (39–43).

It has been hypothesized that changes in chromatin function may serve as one of the basic driving forces for physiological aging (44). We propose that one of the mechanisms underlying decreased chromatin function with age might be altered histone modification patterns. Indeed, histones isolated from various mouse tissues showed a consistent trend toward H4K16 hypoacetylation with increasing age. Similar to this observation, global H4K5 acetylation levels decreased with increasing age in *Caenorhabditis elegans* (43), and H4K12 acetylation decreased in aging mice and caused impaired memory consolidation (45). Consistent with our results, H4K16 acetylation levels were found to be higher in cell lines where life span has been prolonged by ectopic expression of hTERT (46). In contrast to the above studies, H4K16 acetylation levels were shown to increase in a Sir2-dependent manner in yeast replicative aging (47). However, because Sirt1 function in mammalian cells is pleiotrophic and cell type/context-dependent (48, 49), the contribution of Sirt1-dependent deacetylation of H4K16 in mammalian aging is not fully clear. Thus, further studies are required to understand the mechanistic differences between yeast and mammalian aging, with respect to Sirt1 function.

In summary, because lamin A-dependent nuclear defects have been reported in human aging, and prelamin A and progerin have been reported as biomarkers in human skin and vascular aging (29–31), the data presented in this study may have broader sig-

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nificance in understanding the precise role of prelamin A/progerin in physiological aging. Given that H4K16 acetylation is involved in such diverse processes as transcriptional activation, chromatin architecture maintenance, and DNA repair (50–53), it is conceivable that aberrant histone acetylation profiles may have profound cellular consequences in both progeroid and physiological aging. The inherent reversibility of epigenetic modifications also makes histone acetylation a promising target for therapeutic intervention. Undoubtedly, further investigation into how exactly changes in histone acetylation profiles influence aging is likely to yield interesting insights into the epigenetic regulation of aging.

Materials and Methods

Cell Lines and Reagents. MEFs and HEK293 were cultured in complete Dulbecco's Modified Eagle's Medium with 10% FBS. The MOF cDNA was a kind gift from Jerry Workman (Stowers Institute for Medical Research, Kansas City, MO) and was cloned into the pcDNA4/myc-His cloning vector (Invitrogen) to generate the MOF-myc-His plasmid. The carboxy-terminal Ser-Ile-Met of prelamin A was replaced with Phe-Phe-Met A to generate unprocessable prelamin A (14).

Protein Assays. Protein extraction, immunoprecipitation, immunofluorescence, and nuclear matrix isolation details are described in *SI Materials and Methods*. Acid extraction of histones was performed according to Shechter et al. (54). Subcellular fractionation was performed according to Méndez and Stillman (55).

Mice. *Zmpste24*$^{-/-}$ mice (12) were generated and genotyped as described in *SI Materials and Methods*. NaB was mixed in drinking water at the final concentrations of 4 g/L and 8 g/L.

Statistical Analysis. Student's t test was used when comparing two groups. Results for multiple groups were evaluated by ANOVA. Log-rank statistics were used for comparing mice survival curves. Error bars represent SEM.

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