Progerin modulates the IGF-1R/Akt signaling involved in aging

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Progerin, a product of the LMNA mutation, leads to multiple nuclear abnormalities in patients with Hutchinson-Gilford progeria syndrome (HGPS), a devastating premature aging disorder. Progerin also accumulates during physiological aging. Here, we demonstrate that impaired insulin-like growth factor 1 receptor (IGF-1R)/Akt signaling pathway results in severe growth retardation and premature aging in Zmpste24−/− mice, a mouse model of progeria. Mechanistically, progerin mislocalizes outside of the nucleus, interacts with the IGF-1R, and down-regulates its expression, leading to inhibited mitochondrial respiration, retarded cell growth, and accelerated cellular senescence. Pharmacological treatment with the PTEN (phosphatase and tensin homolog deleted on chromosome 10) inhibitor bpV (HOpic) increases Akt activity and improves multiple abnormalities in Zmpste24-deficient mice. These findings provide previously unidentified insights into the role of progerin in regulating the IGF-1R/Akt signaling in HGPS and might be useful for treating LMMA-associated progeroid disorders.

INTRODUCTION

Hutchinson-Gilford progeria syndrome (HGPS) is a rare genetic premature aging disorder that recapitulates many aspects of normal aging (1). In HGPS, a de novo point mutation in the LMNA gene results in the production of a truncated form of the prelamin A precursor, termed as progerin (2, 3), which retains its posttranslational farnesylated tail that is normally cleaved by Zmpste24. Progerin disrupts normal nuclear structural scaffolding by permanently anchoring to the inner nuclear membrane. Mice deficient in Zmpste24 fail to properly cleave prelamin A and show a number of defects resembling HGPS, including severe growth retardation, osteoporosis, alopecia, a generalized failure to thrive, and a markedly shortened life span (4, 5). Efforts to understand how these effects are mediated have largely focused on abnormalities in the nucleus, especially prelamin A–associated genomic instability (6, 7). The importance of genome integrity is underscored by the observation that, in Zmpste24-deficient mice, genetic disruption of the DNA damage response pathway by deletion of the TP53 locus results in improved overall appearance, increased body weight, and extended life span (8). Although improved, mice lacking Zmpste24 and p53 remain substantially smaller than their wild-type (WT) littermates, and the extension of the overall survival is limited. Besides genomic instability, nuclear blebbing is the most prominent cellular phenotype in prelamin A–associated nuclear architecture (9). Although inhibiting prelamin A farnesylation reduces aberrant nuclear shape (10), subsequent treatment with farnesyltransferase inhibitor only moderately ameliorates aging phenotypes in Zmpste24-deficient mice (11). These results strongly suggest that, besides these nuclear abnormalities, additional prelamin A–associated dysregulations undoubtedly exist.

Here, we explore the possible effects of progerin on the somatotroph axis, which diminishes in aging and aging-related disorders (12, 13). We find that progerin accumulates outside of the nucleus and progressively impairs the insulin-like growth factor 1 (IGF-1)/Akt signaling pathway by interacting with IGF-1 receptor (IGF-1R). While Akt1 deficiency aggravates the aging phenotypes in Zmpste24−/− mice, pharmacological treatment with bpV (HOpic) increases Akt activity and significantly improves their postnatal growth and life span. Our results establish an unexpected interaction between progerin and IGF-1R in premature aging, which may help to illuminate the role of naturally occurring progerin and prelamin A during physiological aging (14, 15).

RESULTS

Progerin mislocalizes outside of the nucleus

To explore progerin-associated abnormalities beyond the nucleus, we took advantage of previous observations that progerin mislocalizes into insoluble cytoplasmic aggregates and membranes during mitosis (Fig. S1A) (16, 17). Unexpectedly, we observe interphase cytoplasmic aggregates of progerin in human HGPS fibroblasts (Fig. 1A and fig. S1B). As previous work has shown that inhibiting the lipid modification of the CSIM motif alleviates prelamin A–associated abnormalities in nuclear architecture (9, 10, 16), we wondered whether the farnesylated tail plays any role in the mislocalization of progerin. To address this, we compared the localization of the WT lamin A, prelamin A Δ50 (progerin), prelamin A L647R mutant lacking the Zmpste24 recognition site, and the prelamin A C661S mutant that abolishes farnesylation. In contrast to the notable cytoplasmic aggregates of the prelamin A Δ50 and prelamin A L647R mutants,
which retain their farnesylated C terminus, the prelamin A C661S mutant was localized within the nucleoplasm (fig. S1C). These results indicate that inhibiting the modification of the CSIM motif might be useful for alleviating the cytoplasmic mislocalization of progerin. Given that it has been reported that progerin caused endoplasmic reticulum (ER) stress (18), we made use of ERp19, an ER marker, to find where exactly the cytoplasmic progerin localizes. Consistently, the results show that the aggregates of progerin or prelamin A localize within the ER (Fig. 1B and fig. S1D).

To extend the mislocalization of farnesylated prelamin A, we made use of Zmpste24-deficient mice, a mouse model of progeria. Mice deficient in zinc metalloproteinase Zmspte24 produce prelamin A that cannot undergo the endoproteolytic processing step that yields mature lamin A and share common premature aging
phenotypes with patients with HGPS (4, 5, 11). Both full-length prelamin A and the truncated progerin proteins retain the C terminus farnesylated CSIM motif, leading to permanent anchoring to the inner nuclear membrane, which disrupts normal nuclear structural scaffolding and causes characteristic nuclear blebbing (fig. S2, A and B) (9, 10). Similar to HGPS fibroblasts, interphase cytoplasmic aggregates of prelamin A were observed in Zmpste24−/− mouse embryonic fibroblasts (MEFs) (fig. 1C and fig. S2C). To further validate these findings, we performed Western blot analysis of isolated cytoplasmic proteins from MEFs or transfected cells. Again, we noted the accumulation of prelamin A, lamin C, and progerin in the cytoplasm (Fig. 1, D and E, and fig. S2, D and E). The presence of prelamin A–dependent cytoplasmic lamin C might arise from the interaction between prelamin A and lamin C (19). Furthermore, we also found a marked accumulation of progerin in the cell culture medium of transiently transfected cells (Fig. 1F and fig. S2, F to I). Because progerin is not a classical secretory protein with predictable or known signal sequences, we made use of cell-penetrating peptides (CPPs), which help cellular uptake of the tat protein from HIV (20). We separately deleted two high-scored CPPs in progerin and collected cell culture medium. An obvious reduction of progerin was noted in medium from cells transfected with mutated progerin (Fig. 1G and fig. S2J). We also wondered whether progerin might accumulate in extracellular milieu in a manner of exosome cargo. Exosomes in the cell culture medium were isolated by ultracentrifugation and subsequently identified by traditional negative calnexin and positive CD9 and ALIX (ALG-2 interacting protein X) exosomal markers. However, no progerin was observed in exosomes (fig. S2K), suggesting that extracellular progerin might also arise from cell death after mitotic catastrophe (21).

**Progerin impairs IGF-1/Akt signaling**

The mislocalization of progerin or prelamin A is interesting in light of previous work showing abnormalities beyond the nucleus, including impaired mitochondrial respiration (22) and mammalian target of rapamycin (mTOR) signaling (23) in HGPS fibroblasts or Zmpste24-deficient mice. We thus tested the effects of excreted progerin by treating cells with either conditioned cell culture medium or purified proteins (fig. S2L). We found that progerin entered cells (Fig. 2, A and B, and fig. S3, A and B) and retarded the cell growth of NIH 3T3 cells (Fig. 2C), consistent with similar observations in Zmpste24−/− MEFs (fig. S3C). Moreover, extrinsic progerin treatment also accelerated cellular senescence of WT MEFs, as evidenced by obvious senescence-associated β-galactosidase (SA-β-gal) staining (Fig. 2, D and E) (24). To further validate these effects, we noted a marked elevation of cell cycle inhibitor p21 and p16 in progerin-treated WT MEFs (Fig. 2F), previously noted in Zmpste24−/− MEFs (8). In addition, excreted progerin also impairs mitochondrial respiration and adenosine 5′-triphosphate (ATP) production (Fig. 2, G and H, and fig. S3, D to F) previously reported in HGPS fibroblasts (22).

To understand how progerin causes these alternations, we analyzed the effect of progerin on the IGF-1/Akt signaling that regulates energy metabolism (25) and cell growth (26). Whereas IGF-1 fully activated Akt kinase in WT MEPS, a weakened activation of Akt occurred in progerin-treated cells or Zmpste24−/− MEFs, as evidenced by phosphorylation of Akt at both Thr308 and Ser473 (Fig. 2I). Similar results were obtained using IGF-1–stimulated cells transfected with either WT lamin A or progerin (fig. S4A). Coimmunoprecipitation (Co-IP) assay was subsequently performed to determine whether any physical interaction occurs between progerin and IGF-1R or insulin R (InsR), both of which mediate IGF-1/Akt signaling (27). Interaction between progerin and IGF-1R was noted, in contrast, we detected no interaction between progerin and InsRβ (Fig. 2) and fig. S4, B and C). Direct binding between progerin and the cytoplasmic β subunit of IGF-1R was validated by an in vitro glutathione S-transferase (GST) pull-down assay (Fig. 2K and fig. S4, D and E). Moreover, a similar interaction between prelamin A/C and IGF-1Rβ was observed when endogenous proteins in MEFs were analyzed (fig. S4F). We also found that mature lamin A/lamin C interacts with IGF-1Rβ (fig. S4G), which is consistent with the fact that prelamin A, progerin, and lamin A share common structural features (fig. S4H). By using various lamin A GST constructs in binding assays, we found that the region corresponding to the lamin A C tail is required to mediate binding with IGF-1Rβ (Fig. 2L and fig. S4, I and J).

It was previously shown that prolonged interaction between IGF-1 and IGF-1R down-regulates surface IGF-1R (28). To explore the possible effects of progerin–IGF-1R interaction, we treated cells with either IGF-1 or progerin. Similar to IGF-1, we noted an obvious reduction of IGF-1R in progerin-treated cells (Fig. 2M). Further assessment of Zmpste24-deficient MEFs also revealed a significant decrease in IGF-1R but not InsR (Fig. 2N), both of which mediate IGF-1/Akt signaling. To further validate these findings, we analyzed the tissues of Zmpste24−/− mice, including the heart, muscle, and adipose, which were all previously reported to show abnormalities (4, 5). We noted a marked down-regulation of IGF-1R in these tissues (Fig. 2N). Similar analysis of human cells expressing exogenous progerin further confirmed the decrease in IGF-1R (fig. S4K).

Thus, the observed defects in IGF-1 signaling in Zmpste24-deficient cells appear to be specific to IGF-1R.

**Akt1 deficiency aggravates aging phenotypes in Zmpste24−/− mice**

It is likely that progerin or prelamin A causes cellular dysregulation and aging phenotypes through disruption of crucial IGF-1R signaling pathways. Similar to impaired respiration in HGPS fibroblasts (22), we noted a marked decrease in mitochondrial respiration and ATP production in Zmpste24−/− cells (Fig. 3, A and B, and fig. S5, A to C), which was accompanied by an inhibition of glycolytic function (fig. S5, D and E). Consistent with these metabolic alternations, we found an inhibition of Akt and mTOR kinase that occurred in Zmpste24−/− MEFs (Fig. 3C and fig. S5F). Moreover, decreased activity of Akt/mTOR pathway was evident in the tissues of Zmpste24−/− mice (Fig. 3D and fig. S5G), along with a significant reduction in the overall size and weight of Zmpste24−/− heart and kidney (Fig. 3, E and F). These results underscore the role of Akt/mTOR signaling in the growth of Zmpste24−/− mice.

To assess whether decreased Akt activity might contribute to the various in vivo phenotypic defects observed in Zmpste24−/− mice, we crossed Zmpste24−/− mice with Akt1−/− mice. Previous studies showed that, whereas Akt1-deficient mice are smaller and often experience perinatal death, the remaining Akt1−/− mice continue to grow relatively normally compared to WT animals (29). Statistical analysis of the offspring from intercrosses of Zmpste24−/−Akt1−/− and Zmpste24−/−Akt1−/− cohorts (table. S1). Given the relatively normal appearance of Zmpste24−/− mice compared to WT cohorts before weaning, the overall size of Akt1-deficient mice was smaller, whereas Zmpste24−/−Akt1−/− mice were markedly smaller.
Fig. 2. Progerin/prelamin A down-regulates IGF-1R and impairs IGF-1/Akt signaling. (A) IF analysis of green fluorescent protein (GFP) showing exogenous GFP-progerin (arrows) within NIH 3T3-L1 cells. Scale bar, 10 μm. (B) Flow cytometric analysis of GFP showing the mean fluorescence intensity of NIH 3T3-L1 cells treated with conditioned medium for 3 hours. (C) Growth curves and Western blot analysis of NIH 3T3-L1 cells treated with conditioned medium. (D and E) SA-β-gal staining showing cellular senescence (blue staining) (D) and statistical analysis of SA-β-gal-positive cells from 10 randomly chosen fields (E) of WT MEFs treated with conditioned medium at passage 5. Scale bar, 20 μm. (F) Western blot analysis of WT MEFs treated with conditioned medium at passage 5. (G and H) Mitochondrial respiration (G) and ATP-linked respiration (H) in WT MEFs treated with conditioned medium for 24 hours. (I) Western blot analysis of IGF-1 stimulated activation of Akt in MCF-7 or HEK293T cells treated with progerin or in WT and Zmpste24<sup>−/−</sup> MEFs. (J and K) In vitro interaction between IGF-1Rβ and glutathione S-transferase (GST)–progerin (K) or GST–lamin A truncates (L). (M) Western blot analysis of IGF-1R in HEK293T cells or MCF-7 cells treated with IGF-1 or progerin for 6 hours. (N) Western blot analysis of IGF-1R and InsR in MEFs at passage 4, hearts of 1-month-old WT and Zmpste24<sup>−/−</sup> mice, and muscle and adipose tissue from 2-month-old WT and Zmpste24<sup>−/−</sup> mice. Each lane represents a different mouse. In (G) and (H), OCR, oxygen consumption rate; FCCP, carbonyl cyanide p-trifluoromethoxyphenylhydrazone. In (K) and (L), the asterisks indicate the corresponding protein bands. In (B), (C), (H), and (N), bars indicate means ± SEM, n = 3. **P < 0.01, ***P < 0.001.
Consistent with the overall appearance, a significant decrease in body weight was noted for the double-mutant mice in comparison with \( \text{Zmpste24}^{−/−} \) mice or \( \text{Akt1}^{−/−} \) mice (Fig. 3H). In our colony, the median survival of \( \text{Zmpste24}^{−/−} \text{Akt1}^{+/+} \) mice was 118 days (Fig. 3I). Deletion of \( \text{Akt1} \) combined with \( \text{Zmpste24} \) caused a rough decline of the median life span to 6 days, and essentially all mice succumbed by 129 days. These results show that the absence of \( \text{Akt1} \) shortens the median and maximal life span of mice deficient in \( \text{Zmpste24} \).

**PTEN inhibitor rescues \( \text{Zmpste24}^{−/−} \) mice**

To better understand how progerin might attenuate Akt activity, we analyzed the tumor suppressor PTEN, which acts as the main negative regulator of the Akt kinase (30). We noted an increase in PTEN protein levels in \( \text{Zmpste24}^{−/−} \) MEFs that was further validated in the heart tissue of a 1-month-old \( \text{Zmpste24}^{−/−} \) mice (Fig. 4A). Because our IP analysis revealed no direct interplay between progerin and PTEN (fig. S6A), we sought to take advantage of previously reported regulation of PTEN by transcription factor p53 (31). Because p53 signaling was activated in cells in which p53 expression was decreased by treatment with small interfering RNA (siRNA) (Fig. 4B), this result indicates that prelamin A–associated increase of PTEN might be p53 dependent.

To further underscore the importance of IGF-1/Akt signaling in prelamin A–associated progeria, we treated the \( \text{Zmpste24}^{−/−} \) MEFs and mice with the PTEN inhibitor bpV (HOpic) (32). We found a marked delay of premature senescence in \( \text{Zmpste24}^{−/−} \) cells but not \( \text{Zmpste24}^{+/+} \) cells treated with bpV (HOpic) at a concentration of 20 nM (Fig. 4C). However, inhibition of PTEN did not reduce the levels of misshapen nuclei in \( \text{Zmpste24}^{−/−} \) MEFs (Fig. 4D). After that, 3-week-old \( \text{Zmpste24}^{−/−} \) mice were randomized to treatment with and without bpV (HOpic). We assessed Akt activity in the mice after 1 week of treatment with PTEN inhibitor and found that Akt activity in bpV (HOpic)–treated \( \text{Zmpste24}^{−/−} \) mice was partially restored (Fig. 4E and fig. S6C). Long-term treatment with bpV (HOpic) was associated with a marked improvement in the overall appearance and body weight in both male and female \( \text{Zmpste24}^{−/−} \) mice (Fig. 4, F to H, and fig. S6, D and E). Consistent with this, there

**Fig. 3. Akt1 deficiency aggravates aging phenotypes in \( \text{Zmpste24}^{−/−} \) mice.** (A) Mitochondrial respiration in WT and \( \text{Zmpste24}^{−/−} \) MEFs at passage 4. (B) ATP-linked respiration in WT and \( \text{Zmpste24}^{−/−} \) MEFs at passage 4. (C) Western blot analysis of Akt activity in WT and \( \text{Zmpste24}^{−/−} \) MEFs at passage 4. Each lane represents a different embryo. (D) Western blot analysis of Akt activity in the heart (1-month-old) and kidney (3-month-old, five biological replicates for \( \text{Zmpste24}^{−/−} \)) from WT and \( \text{Zmpste24}^{−/−} \) mice. Each lane represents a different mouse. (E) Overall size of heart and kidney in a 3-month-old WT and \( \text{Zmpste24}^{−/−} \) mice. Scale bar, 3 mm. (F) Weight of heart and kidney in a 3-month-old WT (\( n = 3 \)) and \( \text{Zmpste24}^{−/−} (n = 5) \) mice. (G) Overall appearance of an 8-day-old \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{+/+} \), \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{+/+} \), \( \text{Zmpste24}^{+/+} \) \( \text{Akt1}^{−/−} \), and \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{−/−} \) mice. (H) Body weight of a 4-week-old \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{+/+} \), \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{+/+} \), \( \text{Zmpste24}^{+/+} \) \( \text{Akt1}^{−/−} \), and \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{−/−} \) mice (\( n = 4 \)). (I) Kaplan-Meier survival curves of \( \text{Zmpste24}^{+/+} \) \( \text{Akt1}^{+/+} (n = 6) \), \( \text{Zmpste24}^{+/+} \) \( \text{Akt1}^{+/+} (n = 17) \), \( \text{Zmpste24}^{+/+} \) \( \text{Akt1}^{−/−} (n = 9) \), and \( \text{Zmpste24}^{−/−} \) \( \text{Akt1}^{−/−} (n = 12) \) mice. In (B) to (D), bars indicate means ± SEM, \( n = 3 \); in (F), bars indicate the mean weight of heart or kidney; and in (H), bars indicate the mean body weight of mice for each genotype. **\( P < 0.01 \) and ***\( P < 0.001 \).**
was an obvious increase in the weight of the heart and kidney in bpV (HOpic)–treated Zmpste24−/− mice (Fig. 4I). Similar treatment of WT mice resulted in little difference in the body weight curve of male mice, whereas there was a marked decrease in the female controls (Fig. 4H). In addition, prelamin A–associated abnormal grip strength (5) was also rescued in bpV (HOpic)–treated mice lacking Zmpste24. In addition, our results also demonstrate an obvious delay in the onset of the abnormality and a significant improvement in the overall grip strength (Fig. 4J and fig. S6F).

Fig. 4. PTEN inhibitor rescues Zmpste24−/− mice. (A and B) Western blot analysis of PTEN in MEFs at passage 4 and heart tissue from 1-month-old WT and Zmpste24−/− mice (A) or in HEK293T cells treated with p53 siRNA and subsequently transfected with vector or Flag-progerin for 72 hours (B). (C) SA-β-gal staining showing cellular senescence of WT or Zmpste24−/− MEFs treated with PBS or bpV (HOpic) at passage 5. Scale bar, 20 μm. KO, knockout. (D) IF analysis of characteristic nuclear blebbing in WT or Zmpste24−/− MEFs treated with PBS or bpV (HOpic) at passage 5. Scale bar, 10 μm. (E) Western blot analysis of Akt activity from WT and Zmpste24−/− mice treated with bpV (HOpic) or untreated (two biological replicates). (F) Overall appearance of a 3-month-old male WT and Zmpste24−/− mice treated with bpV (HOpic) or untreated (two biological replicates). (G and H) Body weight curves of male Zmpste24+/+ (vehicle, n = 8), Zmpste24+/− (bpV, n = 7), Zmpste24−/− (vehicle, n = 14), and Zmpste24−/− (bpV, n = 8) mice (G) or female Zmpste24+/+ (vehicle, n = 11), Zmpste24+/− (bpV, n = 7), Zmpste24−/− (vehicle, n = 14), and Zmpste24−/− (bpV, n = 7) mice (H) (means ± SEM). (I) Weight of heart and kidney in a 3-month-old Zmpste24+/+ (bpV, n = 5) and Zmpste24−/− (vehicle, n = 5) and 4-month-old Zmpste24−/− (bpV, n = 4) mice. (J) Kaplan-Meier plot showing the percent of Zmpste24−/− (vehicle, n = 7) and Zmpste24−/− (bpV, n = 11) mice with normal grip strength. (K) Kaplan-Meier survival curves of Zmpste24−/− (vehicle, n = 27) and Zmpste24−/− (bpV, n = 15) mice. (L) Correlation analysis between maximal body weight and life span in Zmpste24−/− mice (n = 69). In (C) and (D), cells examined from five randomly chosen fields of view for each group are recorded. In (A), (G), and (H), bars indicate means ± SEM, n = 3. *P < 0.05, **P < 0.01, and ***P < 0.001. N.S., not significant.
On the basis of these observations, we asked whether the PTEN inhibitors could extend the life span of Zmpste24−/− mice. In our colony, the median survival of Zmpste24−/− mice treated with vehicle was only 74 days, ranging from 21 to 143 days (Fig. 4K). Treatment with PTEN inhibitors provided a survival advantage and increased the median life span of Zmpste24−/− mice to 131 days (Fig. 4K and fig. S6G). Given the obvious increase in the body weight followed by a 77% extension of median life span of bpV (HOpic)–treated Zmpste24−/− mice (Fig. 4, G, H, and K) and a corresponding 95% reduction of life span in mice lacking both Zmpste24 and Akt1 (Fig. 3, H and I), we hypothesized that life span and body weight would show a significant correlation. Subsequent statistical analysis suggested that survival was positively correlated with maximal body weight in mice deficient in Zmpste24 (Fig. 4L), further substantiating the effect of diminished Akt activity on Zmpste24-deficient phenotype.

**DISCUSSION**

Mice deficient in Zmpste24 are totally normal at birth but experience rapid growth retardation after weaning and are subject to accelerated aging until death in about 5 to 6 months (4, 5). Our previous work demonstrated that activation of Akt/mTOR signaling guarantees normal embryonic development and birth in Zmpste24−/− mice (fig. S7, A and B), as evidenced by frequent embryonic lethality in Zmpste24-deficient mice with genetic reduction of mTOR (33). However, a marked inhibition of mTOR kinase is obvious in the heart, muscle (22), and lung tissue of adult Zmpste24−/− mice (fig. S7C). A recent study also showed a reduction of mTOR activity in the G608G transgenic mouse line, a mouse model of HGPS (34). It has remained unknown what causes the progressive attenuation of Akt activity in Zmpste24-deficient mice with genetic reduction of mTOR, which might involve in diminished IGF-1/Akt signaling. In our study, we showed that cytoplasmic progerin down-regulates IGF-1R and impairs IGF-1/Akt signaling. These results underscore the importance of IGFr1 signaling in regulating Akt activity to maintain organismal homeostasis in prelamin A–associated disorders and thus provide previously unidentified insight into the role of naturally occurring progerin in physiological aging.

**MATERIALS AND METHODS**

**Mice**

All protocols used for animal studies were approved by the Institutional Animal Care and Use Committee of China Medical University (approval ID no. CMU2017343). Zmpste24−/− and Akt1+/− mice were described previously (5, 29). Zmpste24−/− C57BL/6J mice and Akt1+/− 129S4/SvJae mice were crossed to generate Zmpste24−/−Akt1+/− pups on a mixed genetic background (=50% C57BL/6J and =50% 129S4/SvJae). Subsequently, an intercross was carried out with double heterozygous to acquire Zmpste24−/− and Zmpste24−/− mice with various Akt1 genetic status. For the intraperitoneal injection of bpV (HOpic), we randomized Zmpste24−/− and Zmpste24−/− mice on a C57BL/6J background to phosphate-buffered saline (PBS) or PBS containing bpV (HOpic). Littermate controls were used for analysis, and all mice were genotyped routinely by polymerase chain reaction with mouse tail DNA. Except for newborn animals, biological analysis of the heart, liver, kidney, lung, muscle, and adipose was performed for both male and female mice at 1 to 3 months of age. The number of surviving mice was recorded every day, and the body weight and normal grip strength of male and female mice were recorded twice per week. Abnormal grip strength of hind limbs of mice was tested according to previously described protocols (5).

**Cells**

Primary normal human dermal fibroblasts isolated from a healthy female donor (aged 24 years) (42) and HGPS dermal fibroblasts HGADFN143 (HG143) (6) were described previously. MCF-7 (Michigan Cancer Foundation 7) cells, human embryonic kidney (HEK) 293T cells, HeLa cells, and MEFs were described previously (33). Mouse fibroblast NIH 3T3-L1 cells were cultured in Dulbecco’s modified Eagle’s medium (DMEM) supplemented with 10% fetal bovine serum (FBS). All cells were cultured in medium supplemented with penicillin (100 U/ml) and streptomycin (100 μg/ml) in an atmosphere of 5% CO2 at 37°C.

For proliferation analysis, NIH 3T3-L1 cells were plated at a density of 1 × 104 cells per well in 96-well plates. Cells were cultured in DMEM containing 10% FBS with vector or progerin conditional medium (50%) and changed every day. Cell density was measured using a Cell Counting Kit-8 (CCK8) assay.

For cellular senescence, MEFs derived from E14.5 (embryonic day 14.5) embryos were plated at a density of 5 × 104 cells per well
in six-well plates. MEFs were cultured in medium DMEM containing 15% FBS with vector or progerin conditional medium (50%). Every 3 days, cells were trypsinized, counted, and plated again at the same density.

**Plasmids**

Human prelamin A WT (lamin A), prelamin A Δ50 (progerin), prelamin A L647R, and prelamin A C661S were separately cloned into an empty pCDNA4.0-Flag vector and transfected into cells using Higene (Applygen) according to the manufacturer’s instructions. Cells or medium were harvested at 48 or 72 hours as indicated after transfection. GST, GST–progerin, GST–IGF-1Rβ, and GST–lamin A truncates indicated in Fig. S3I were constructed using the pGEX-5X-1 vector. All constructs in this study were verified by DNA sequencing. p53 siRNA was purchased from Ribobio Co. Ltd., Guangzhou, China. The p53 targeting sequence was 5′-AGATGGCCCATGGCCGAC-3′.

**Reagents and antibodies**

IGF-1 was purchased from Cell Signaling Technology (no. 8917). bpV (HOpic) was from Selleck Chemicals (S8651). The following antibodies were purchased from Cell Signaling Technology: IGF-1Rβ (no. 9750), InsRβ (no. 23413), Akt (no. 4691), phospho–Akt–Thr58 (no. 13038), phospho–Akt–Ser473 (no. 4060), PTEN (no. 9188), mTOR (no. 2983), phospho–mTOR–Ser2448 (no. 5536), S6K1 (no. 2708), and phospho–S6K1–Thr389 (no. 9234). Antibody against β-actin was purchased from ABLonal. Lamin A/C (sc-20681), progerin (sc-81611), Erp19 (sc-376410), p53 (sc-53394), p21 (sc-6246), and p16 (sc-377412) were purchased from Santa Cruz Biotechnology. Flag-tag antibody (SG4110-16) was from Shanghai Genomics Technology. Anti-Flag affinity gel (B231201) was from Bimake. The anti–prelamin A (MABT858) and FLAG peptide (F3290) were from Sigma–Aldrich.

**Immunofluorescence**

Human fibroblasts, MEFs, and MCF-7 cells were mounted on glass slides. Cells were washed three times with PBS and then fixed with ice-cold 4% paraformaldehyde (PFA) for 15 min, followed by washing with PBS. Cells were permeabilized with 0.25% Triton X-100 for 15 min. Cells were then blocked with 5% bovine serum albumin for 1 hour at room temperature and incubated with indicated primary antibodies overnight at 4°C. After washing three times with PBS, cells were incubated with the corresponding fluorescent secondary antibodies (Alexa Fluor–conjugated secondary antibody, 1:500; Life Technologies) for 1 hour at room temperature in the dark. The nucleus was stained with 4′,6-diamidino-2-phenylindole at room temperature for another 20 min. Images were captured using a confocal microscope system (Nikon).

**Fluorescence-activated cell sorting analysis**

For the presence of exogenous green fluorescent protein (GFP)–progerin within NIH 3T3-L1 cells, cells were seeded in a 12-well plate at a density of 1 × 10^4 per well and allowed to grow to 70 to 80% confluence. These cells were then incubated with conditioned medium containing Flag-vector or Flag-GFP-progerin for 3 hours. Cells were harvested by centrifugation at 200g for 5 min and incubated with 0.125% (w/v) trypsin blue for 1 min to quench extracellular fluorescence (43). The intensity of fluorescence associated with intracellular GFP protein was analyzed by flow cytometry.

**Bacteria expression and GST pull-down**

GST–tagged constructs were transformed into *Escherichia coli* BL21, and proteins were purified with glutathione sepharose 4B according to the manufacturer’s protocol. Cell-free in vitro translation was performed using the transcription and translation kit in vitro (Promega, P2221), following the manufacturer’s guidance. In vitro–translated proteins were then subjected to GST pull-down with GST or GST fusion proteins following an overnight incubation at 4°C in binding buffer (20 mM tris–HCl at pH 7.5, 50 mM NaCl, 10% glycerol, and 1% NP-40). The product was washed three times with binding buffer and then subjected to immunoblot analysis.

**Western blot analysis and IP**

Western blot analysis was performed using standard procedures for whole-cell extracts. Cells were lysed for 30 min on ice with IP lysis buffer [50 mM tris–HCl (pH 7.4), 150 mM NaCl, 0.25% sodium deoxycholate, 1% NP-40, 1 mM EDTA, and 1% Triton X-100] supplemented with protease and phosphatase inhibitor cocktails, and the total protein was harvested by cryogenic centrifugation at 13,500g for 20 min. Protein concentration was measured by G250, and 40 μg of cell lysate was used in samples. Protein was also isolated from in vivo sources. The fresh tissues were dissected from mice soon after death. Tissues were immediately frozen in liquid nitrogen and incubated with lysis buffer containing protease and phosphatase inhibitor cocktails to prepare tissue homogenate. Samples were placed on ice for 30 min with shaking to solubilize the tissue homogenate. The homogenate was centrifuged at 13,500g at 30 min at 4°C. The supernatant was transferred to a new tube, and the protein concentration was measured by G250. Tissue lysate (40 μg) was prepared for protein separation and immunoblot analysis.

Subcellular fractionation was performed as previously described (44). For Co-IP analysis, cells were lysed with IP lysis buffer, and cell lysates were incubated with the indicated antibody [mouse immunoglobulin (IgG; 2 μg/ml), lamin A/C (1.5 μg/ml), flag (2 μg/ml), and progerin (2 μg/ml)] at 4°C for 24 hours followed by the addition of 20 μl of protein A/G beads (Santa Cruz Biotechnology) for another 2 hours at 4°C. For medium IP or cytoplasm IP, the indicated antibody [mouse IgG (2 μg/ml), lamin A/C (1.5 μg/ml), flag (2 μg/ml), and progerin (2 μg/ml)] was added and incubated at 4°C overnight followed by the addition of 20 μl of protein A/G beads for another 2 hours at 4°C. Anti-Flag affinity gel was added in whole-cell lysis (for Co-IP analysis, 20 μl/ml, 24 hours), cytoplasm (cytoplasm IP, 20 μl/ml, overnight), or medium (medium IP, 20 μl/10 ml, overnight) and incubated at 4°C. After IP, the samples were washed with IP lysis buffer for three times. Proteins were eluted with 2× SDS sample buffer. The eluates were subjected to SDS–polyacrylamide gel electrophoresis, and proteins were detected by immunoblot.

**Flag-progerin fusion protein purification**

Total protein of HEK293T cells transfected with Flag-vector or Flag-progerin was harvested as indicated in IP and incubated with anti-Flag affinity gel according to the manufacturer’s protocol. After that, the FLAG peptide was added to anti-Flag affinity gel to elute the bound FLAG fusion protein, following the manufacturer’s guidance. Purified protein was identified by Coomassie brilliant blue staining.

**CCK8 assay**

Cultured cells (1 × 10^4 cells per well) were seeded into 96-well plate. After incubation, 10 μl of CCK8 solution (Dojindo Molecular
Cell proliferation analysis
MEFs derived from E14.5 embryos were plated at a density of 5 × 10^3 cells per well in six-well plates. Every 3 days, cells were trypsinized, counted, and plated again at the same density. Three embryos were used representing each genotype.

Glycolytic function and mitochondrial respiration
The extracellular acidification rate (ECAR) and oxygen consumption rate (OCR) were measured using a Seahorse XFp Analyzer (Seahorse Bioscience, 103020-100). For WT and Zmpste24−/− MEFs at passage 4, cells were plated on Seahorse XFp plates for 12 hours at a concentration of 8 × 10^3 cells per well and then washed and incubated with assay medium at 37°C for 1 hour in a non-CO2 incubator. For ECAR, glucose, oligomycin, and 2-deoxyglucose were injected into the medium at final concentrations of 10 mM, 1 mM, and 50 mM, respectively. For OCR, carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), oligomycin, and Rotenone/antimycin A were injected into the medium at final concentrations of 1, 1.5, and 0.5 μM, respectively. The ECAR and OCR were automatically recorded and calculated by the Seahorse XFp Analyzer software following the manufacturer’s recommendation.

For WT MEFs at passage 2, cells were plated on Seahorse XFp plates and treated with vector or progerin conditioned medium for 24 hours at a density of 1 × 10^4 cells per well and then washed and incubated with assay medium at 37°C for 1 hour in a non-CO2 incubator. For ECAR, glucose, oligomycin, and 2-deoxyglucose were injected into the medium at final concentrations of 10 mM, 1 μM, and 50 mM, respectively. For OCR, FCCP, oligomycin, and rotenone/antimycin A were injected into the medium at final concentrations of 1, 1, and 0.5 μM, respectively. The ECAR and OCR were automatically recorded and calculated by the Seahorse XFp software following the manufacturer’s recommendation.

SA-β-gal assay
SA-β-gal activity was assessed with a senescence β-galactosidase staining kit (Beyotime Biotechnology, C0602), according to manufacturer’s protocol. MEFs were seeded in six-well plates. MEFs were washed three times with PBS and fixed with 4% PFA at room temperature for 15 minutes. After washing with PBS, cells were stained with 1 ml of freshly prepared β-gal detection solution at 37°C overnight. The cells were washed twice with PBS and overlaid with PBS. Images were captured under a microscope (Nikon). Blue-stained MEFs were indicated for cellular senescence.

Statistics analysis
All experiments were carried out with at least three replicates. For experiments using mice or MEFs, at least three biological replicates were analyzed, unless stated otherwise. The statistical analysis was carried out by using GraphPad Prism software v.5.01 with log rank (Mantel-Cox) test, Student’s t test, one-way analysis of variance (ANOVA) coupled with Tukey’s post hoc test, and two-way ANOVA coupled with Bonferroni post hoc tests. Significance was considered when the P value was less than 0.05. Respective P values as a measure of significance are indicated.

REFERENCES AND NOTES
1. M. A. Merideth, L. B. Gordon, S. Clauss, V. Sachdev, A. C. M. Smith, M. B. Perry, C. C. Brewer, C. Zaleski, H. J. Kim, B. Solomon, B. P. Brooks, L. H. Gerber, M. L. Turner, D. L. Domingo, T. C. Hart, J. Graf, J. C. Reynolds, A. Gropman, J. A. Yanovski, M. Gerhard-Herman, F. S. Collins, E. G. Nabel, R. D. Cannon III, W. A. Gahl, W. J. Instron, Phenotype and course of Hutchinson-Gilford progeria syndrome. N. Engl. J. Med. 358, 592–604 (2008).
2. M. Eriksson, W. T. Brown, L. B. Gordon, M. W. Glynn, J. Singer, L. Scott, M. R. Erdos, C. M. Robbins, T. Y. Moses, P. Berglund, A. Dutta, E. Pak, S. Durbin, A. B. Csoka, M. Boehnke, T. W. Glover, F. S. Collins, Recurrent de novo point mutations in lamin A cause Hutchinson-Gilford progeria syndrome. Nature 423, 293–298 (2003).
3. A. De Sandre-Giovannoli, R. Bernard, P. Cau, C. Navarro, J. Amiel, I. Boccaccio, S. Lyonnnet, D. J. Chen, D. Pei, A. M. Pendás, J. Cadiñanos, C. López-Otín, Defective prelamin A processing and muscular and adipocyte alterations in Zmpste24 metalloproteinase-deficient mice. Nat. Genet. 31, 94–99 (2002).
4. M. O. Bergo, B. Gavin, J. Ross, W. K. Schmidt, C. Hong, L. V. Kendall, A. Mohr, M. Meta, H. Genant, Y. Jiang, E. R. Wisner, N. van Bruggen, R. A. D. Carano, S. Michaelis, S. M. Griffin, S. G. Young, Zmpste24 deficiency in mice causes spontaneous bone fractures, muscle weakness, and a prelamin A Processing defect. Proc. Natl. Acad. Sci. U.S.A. 99, 13049–13054 (2002).
5. B. Liu, J. Wang, K. M. Chan, W. M. Tia, W. Deng, X. Guan, J. D. Huang, K. M. Li, P. Y. Chau, D. J. Chen, D. Pei, A. M. Pendás, J. Cadiñanos, C. López-Otín, H. F. Tse, C. Hutchison, J. Chen, Y. Cao, K. S. E. Cheah, K. Tryggvason, Z. Zhou, Genomic instability in laminopathy-based premature aging. Nat. Med. 11, 780–785 (2005).
6. B. C. Capell, M. R. Erdos, J. P. Madigan, J. J. Fiordalisi, R. Varga, K. N. Conneely, L. B. Gordon, C. J. der, A. D. Cox, F. S. Collins, Inhibiting farnesylase of progerin prevents the characteristic nuclear blebbing of Hutchinson-Gilford progeria syndrome. Proc. Natl. Acad. Sci. U.S.A. 102, 12879–12884 (2005).
7. J. J. Toth, S. H. Yang, X. Qiao, A. P. Beigneux, M. H. Gelb, C. L. Moulson, J. H. Miner, S. G. Young, L. G. Fong, Blocking protein farnesyltransferase improves nuclear shape in fibroblasts from humans with progeroid syndromes. Proc. Natl. Acad. Sci. U.S.A. 102, 12873–12878 (2005).
8. L. G. Fong, D. Frost, M. Meta, X. Qiao, S. H. Yang, C. Coffinier, S. G. Young, A protein farnesyltransferase inhibitor ameliorates disease in a mouse model of progeria. Science 311, 1621–1623 (2006).
9. D. Rudman, A. G. Feller, H. S. Nagara, G. A. Gergans, P. Y. Lalitha, A. F. Goldberg, R. A. Schlenker, L. Cohn, I. W. Rudman, D. E. Mattson, Effects of human growth hormone in men over 60 years old. N. Engl. J. Med. 323, 1–6 (1990).
10. J. Chen, E. Yuday, B. Bell, M. A. Lobeg, S. Beans, D. Dr. Filippi, J. T. Browde, Decline in IGF1 in the bone marrow microenvironment initiates hematopoietic stem cell aging. Cell Stem Cell 28, 1473–1482.e7 (2021).
11. P. Scaffidi, T. Misteli, Lamin A-dependent nuclear defects in human aging. Science 312, 1059–1063 (2006).
12. J. Skepper, D. T. Warren, Y. Liu, R. M. Nair, T. Tajisc, N. Figg, R. Shroff, J. Skepper, C. M. Shanahan, Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. Circulation 121, 2200–2210 (2010).
13. K. Cao, B. C. Capell, M. R. Erdos, K. Djalali, F. S. Collins, A lamin A protein isoform overexpressed in Hutchinson-Gilford progeria syndrome interferes with mitosis in progeria and normal cells. Proc. Natl. Acad. Sci. U.S.A. 104, 4949–4954 (2007).
14. T. Decat, T. Shimi, S. A. Adam, A. E. Ruisinol, D. A. Andres, H. P. Spielmann, M. S. Sinensky, R. D. Goldman, Alterations in mitosis and cell cycle progression caused by a mutant lamin A known to accelerate human aging. Proc. Natl. Acad. Sci. U.S.A. 104, 4955–4960 (2007).
15. M. R. Hamczyk, R. Villa-Bellosa, V. Quesada, P. Gonzalez, S. Vidak, R. M. Nevado, M. J. Andres-Manzano, T. Misteli, C. López-Otín, V. Andrés, Progerin accelerates atherosclerosis by inducing endoplasmic reticulum stress in vascular smooth muscle cells. EMBO Mol. Med. 11, e9732 (2019).
16. Q. Ye, H. J. Warren, Protein–protein interactions between human nuclear laminas expressed in yeast. Exp. Cell Res. 219, 292–298 (1995).
20. A. D. Frankel, C. O. Pabo. Cellular uptake of the tat protein from human immunodeficiency virus. Cell 55, 1189–1193 (1988).
21. H. Zhang, Z.-M. Xiong, K. Cao. Mechanisms controlling the smooth muscle cell death in progeria via down-regulation of poly(ADP-ribose) polymerase 1. Proc. Natl. Acad. Sci. U.S.A. 111, E2261–E2270 (2014).
22. J. Rivera-Torres, R. Acín-Perez, P. Cabezás-Sánchez, F. G. Osorio, C. González-Gómez, D. Megas, C. Cámara, C. López-Otín, J. A. Enriquez, J. L. Luque-García, V. Andréis. Identification of mitochondrial dysfunction in Hutchinson-Gilford progeria syndrome through use of stable isotope labeling with amino acids in cell culture. J. Proteomics 91, 466–477 (2013).
23. G. Mariño, A. P. Ugalde, N. Salvador-Montoliu, I. Varela, P. M. Quirós, J. Cadiñanos, I. van der Pluijm, J. M. P. Freije, C. López-Otín. Premature aging in mice activates a systemic metabolic response involving autophagy induction. Hum. Mol. Genet. 17, 2196–2211 (2008).
24. G. P. Dimri, X. Lee, G. Basile, M. Acosta, G. Scott, C. Roskelley, E. E. Medrano, M. Linskens, I. Rubelj, O. Pereira-Smith. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. U.S.A. 92, 9363–9367 (1995).
25. J. T. Cunninghamham, J. T. Rogers, D. H. Araw, F. Vazquez, V. K. Mootha, P. Puigserver, mTOR controls mitochondrial oxidative function through a YY1-PGC-1alpha transcriptional complex. Nature 450, 736–740 (2007).
26. H. Cho, J. L. Thorvaldsen, Q. Chu, F. Feng, M. J. Birnbaum. Akt1/PKBalpha is required for normal growth but dispensable for maintenance of glucose homeostasis in mice. J. Biol. Chem. 278, 38349–38352 (2001).
27. D. R. Alessi, M. Andjelkovic, B. Caudwell, P. Cron, N. Morrice, P. Cohen, B. A. Hemmings. Mechanism of activation of protein kinase B by insulin and IGF-1. EMBO J. 15, 6541–6551 (1996).
28. M. A. de Vroe, J. A. Romanus, M. L. Staandt, R. J. Pollet, S. P. Nisley, M. M. Rechler. Interaction of insulin-like growth factors with a nonfusing mouse muscle cell line: Binding, action, and receptor down-regulation. Endocrinology 114, 1917–1924 (1984).
29. Z. Z. Yang, O. Tschopp, M. Hemmings-Miesczak, J. Feng, D. Brodbeck, E. Perentes, B. A. Hemmings. Protein kinase B alpha Akt1 regulates placental development and fetal growth. J. Biol. Chem. 278, 32124–32131 (2003).
30. X. Wu, K. Senechal, M. S. Nesbit, Y. E. Whang, C. L. Sawyer, The PTEN/MMAC1 tumor suppressor phosphatase functions as a negative regulator of the phosphoinositide 3-kinase-Akt pathway. Proc. Natl. Acad. Sci. U.S.A. 95, 15587–15591 (1998).
31. V. Stambolic, D. MacPherson, D. Sas, Y. Liu, B. Snow, Y. Jang, S. Benchimol, C. Wiel, M. O. Berge, Targeting RAS-converting enzyme 1 overcomes senescence and improves progeria-like phenotypes of ZMPSTE24 deficiency. Aging Cell 19, e13200 (2020).
32. G. Mariño, A. P. Ugalde, A. F. Fernández, F. G. Osorio, A. Fueyo, J. M. P. Freije, C. López-Otín. Insulin-like growth factor I treatment extends longevity in a mouse model of human premature aging by restoring somatotroph axis function. Proc. Natl. Acad. Sci. U.S.A. 107, 16268–16273 (2010).
33. M. Wang, L. Wang, M. Qian, J. Tang, Z. Liu, Y. Lai, Y. Ao, Y. Huang, Y. Meng, L. Shi, P. Cau, X. Cao, Z. Wang, B. Qin, B. Liu. PML2-mediated thread-like nuclear bodies mark late senescence in Hutchinson-Gilford progeria syndrome. Aging Cell 19, e13147 (2020).
34. H. Yao, X. Chen, M. Kashif, T. Wang, M. X. Ibrahim, E. Tüksammel, G. Revéchéon, M. Eriksson, C. Wiel, M. O. Berge, Targeting RAS-converting enzyme 1 overcomes senescence and improves progeria-like phenotypes of ZMPSTE24 deficiency. Aging Cell 19, e13147 (2020).
35. H. Park, Y. Li, R. W. Tsien. Influence of synaptic vesicle position on release probability and exocytotic fusion mode. Science 335, 1362–1366 (2012).
36. J. Wyssok, P. T. Reilly, W. Herr, Loss of HCF-1-chromatin association precedes temperature-induced growth arrest of tsBN67 cells. Mol. Cell. Biol. 21, 3820–3829 (2001).

Acknowledgments: We are grateful to S. G. Young for providing Zmpste24−/− mice, to Z. Z. Yang and D. H. Chen for Akt1+/− mice, and to Z. X. Xiao for IGF-1R plasmid. Funding: This work was supported by the Key project of the National Natural Science Foundation 82030091 (to L.C.), National Key R&D Program of China 2016YFC1302400 (to L.C.), Ministry of Education Innovation team development plan IRT_17R107 (to L.C.), and Key project of Liaoning Science Foundation 2019JH210300 and 21Z1-4-021 (to L.C.). Author contributions: B.J. and L.C. designed the research. B.J. and X.W. performed most of the research. B.J. and X.W. performed the Western blot, IP, and GST pull-down experiments. X.W., F.M., I.S., and S.C. performed the plasmids constructs and immunofluorescence analysis. B.J. and X.W. analyzed and presented the results of the mouse model. X.W. performed the proliferation analysis and flow cytometry analysis. H.S. isolated exosomes. Y.D. and M.L. performed the senescence assay. H.X., N.B., G.Q., X.Y., W.G., F.Y., T.Z., X.M.L., Y.F., Z.W., Y.G., M.M., D.Z., J.L., X.N.L., W.Z., and B.L. contributed new reagents or analytic tools. B.J. wrote the paper. T.F. and L.C. contributed to editing the manuscript. Competing interests: The authors declare that they have no competing interests. Data and materials availability: All data needed to evaluate the conclusions in the paper are present in the paper and/or the Supplementary Materials.

Submitted 8 January 2022 Accepted 24 May 2022 Published 8 July 2022 10.1126/sciadv.abo0322