Hutchinson-Gilford progeria syndrome (HGPS) is a rare autosomal dominant genetic disease that is caused by a silent mutation of the LMNA gene encoding lamins A and C (lamin A/C). The G608G mutation generates a more accessible splicing donor site than does WT and produces an alternatively spliced product of LMNA called progerin, which is also expressed in normal aged cells. In this study, we determined that progerin binds directly to lamin A/C and induces profound nuclear aberrations. Given this observation, we performed a random screening of a chemical library and identified 3 compounds (JH1, JH4, and JH13) that efficiently block progerin–lamin A/C binding. These 3 chemicals, particularly JH4, alleviated nuclear deformation and reversed senescence markers characteristic of HGPS cells, including growth arrest and senescence-associated β-gal (SA–β-gal) activity. We then used microarray-based analysis to demonstrate that JH4 is able to rescue defects of cell-cycle progression in both HGPS and aged cells. Furthermore, administration of JH4 to Lmna<sup>G609G/G609G</sup> mutant mice, which phenocopy human HGPS, resulted in a marked improvement of several progeria phenotypes and an extended lifespan. Together, these findings indicate that specific inhibitors with the ability to block pathological progerin–lamin A/C binding may represent a promising strategy for improving lifespan and health in both HGPS and normal aging.
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Despite several reports dealing with the role of this protein in cell-cycle regulation (11, 17, 18) and senescence (12, 19), how progerin induces various cellular defects and premature aging remains to be revealed. Remarkably, proteomic studies and 2-hybrid approaches have identified lamin A/C as a progerin-binding protein (20, 21). On this basis, we hypothesized that the interaction of progerin with lamin A/C contributes to the development of the senescence phenotype of HGPS and aged cells.

Results
Direct interaction between progerin and lamin A/C but not lamin B. To investigate our hypothesis, we performed a binding assay and found that C-terminal progerin and lamin A associated with lamin A and C but not with lamin B1 (Figure 1A and Supplemental Figure 1A; supplemental material available online with this article; doi:10.1172/JCI84164DS1). In addition, the binding affinity between progerin and lamin A/C was stronger than that between lamin A/C and lamin A/C (Figure 1B and Supplemental Figure 1B). To dissect the progerin domain involved in lamin A/C binding, we performed a glutathione-S-transferase pull-down (GST pull-down) assay using a His-tagged lamin A N-terminal region (LMNA-N; aa residues 1-300) and a lamin A middle region (LMNA-M; aa residues 301-564) with bead-coupled GST–C-terminal fragments of lamin A (GST-LMNA) or progerin (GST-progerin). We found that the middle region of lamin A was responsible for progerin binding (Figure 1, C and D). We could confirm the direct interaction of both proteins through Far Western blotting.
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Figure 1. Identification of a lamin A-progerin-binding inhibitor. (A) Interaction of progerin and LMNA A/C but not lamin B. GST pull-down assay using lysates from HEK293 cells transfected with lamin A, B, C, and progerin after incubation with the C-terminal 100-aa sequence of lamin A (564–664 aa; GST-LMNA) or 50-aa sequence of progerin (564–614 aa; GST-progerin). (B) Strong interaction between lamin A and progerin. Lamin A-progerin interaction was detected after a 1-hour incubation. However, progerin did not show evidence of self-association. Rx, treatment. (C) Direct interaction between lamin A and progerin. In vitro–binding assay using His-tagged N-terminal (1–300 aa; LMNA-N) and the middle region (LMNA-M) of lamin A after incubation with GST-LMNA or GST-progerin. Associated proteins were analyzed by Western blotting. N, LMNA-N; M, LMNA-M. (D) Scheme of the interaction between lamin A and progerin. (E) ELISA results. His-tagged LMNA-M was fixed in a 96-well plate and incubated with GST-LMNA or GST-progerin. Photograph of the 96-well plate (inset) and ELISA value. (F and G) Progerin did not induce nuclear deformation in neuron or embryonic neuron cells. Transfection of GFP-progerin or lamin A alone into the neuroblastoma cell line SH-SY-5Y (F) and the mouse neuroembryonic cell line N2A did not induce nuclear deformation. However, cotransfection could induce it. Cells were transfected with the indicated vectors for 24 hours. For visualization of the nuclear membrane, cells were stained with emerin (red) and DAPI for DNA (blue). Scale bar: 10 μm.

analysis (Supplemental Figure 1C). Considering that progerin can strongly interact with LMNC (which corresponds to aa 1–572 of LMNA), we excluded the possibility that the C-terminal extension region of LMNA is a target of progerin. By ELISAs with an immobilized LMNA-M domain and the GST-fused recombinant lamin A or progerin, we could estimate that progerin–lamin A binding affinity is more than 2-fold stronger than that of lamin A–lamin A binding (Figure 1E). Considering that lamin A and C form intermediate filaments by head-to-tail binding and bundle through the C-terminal and middle regions (22), progerin may induce a stronger or tighter but irregular bundle formation than does lamin A or C alone. This hypothesis was consistent with the HGPS cell phenotype, in which lamin A and C formed an irregular fiber arrangement (Supplemental Figure 1, D and E). Indeed, progerin alone did not induce nuclear deformation in lamin A/C-deficient neuron or embryonic neuron cells, whereas cotransfection with lamin A could induce it (Figure 1, F and G, and Supplemental Figure 1, F and G), despite low expression of progerin in N2A cells (Supplemental Figure 1G). These results indicated that nuclear lamina alteration can derive from the strong binding of progerin to lamin A and C.

Screening of specific binding inhibitor of progerin–lamin A binding. The above findings prompted us to search for putative progerin/lamin A-binding inhibitors by using an ELISA-based screening system. In brief, progerin, immobilized in 96-well plates, was incubated sequentially with a chemical library and His–lamin A (Supplemental Figure 2, A and B). This screening identified 5 chemicals that blocked this interaction (JH1, JH4, JH13, LG-02, and KEK-2-24, Supplemental Figure 2C). Because KEK-2-24 is a mixture of compounds from Panax ginseng and LG-02 requires complicated synthetic steps, we excluded them from further study. A detailed synthetic pathway for the selected chemicals is described in the Chemical Synthesis section of the Supplemental Methods. Interestingly, in spite of the blind screening, the selected chemicals were structurally similar (Supplemental Figure 2C) and showed very low cytotoxicity (Supplemental Figure 2D). Therefore, we decided to further investigate the activity of JH chemicals, particularly JH4, because JH1 and JH13, despite their similar effects, show very low solubility. We first confirmed their inhibitory effect of the JH chemicals on the binding between progerin and lamin A through in vitro binding assays (Supplemental Figure 2E). Indeed, less than 1 μM of JH4 (IC50 = 0.65 μM) could block 50% of the interaction between progerin and lamin A without disruption of lamin A self-association (Supplemental Figure 2E).

The effect of progerin–lamin A–binding inhibitors in HGPS model cells. To confirm the inhibitory effect of JH chemicals on binding between progerin and lamin A, we performed the GST pull-down assay and observed that JH4 blocked the interaction of GST–lamin A with GFP-progerin, but not with GFP–lamin A, in a GST pull-down assay using cell lysates (Figure 2A and Supplemental Figure 3A). However, the farnesyltransferase inhibitor FTI-277, known to ameliorate nuclear deformation (23, 24), did not show an obvious effect on progerin–lamin A binding (Figure 2A and Supplemental Figure 3B). Next, we checked their in vivo effect in progerin and GFP–lamin A–transfected HEK293 cells through IP assays with an anti-GFP Ab. The binding between progerin and lamin A was markedly reduced by the JH chemicals, while the binding between lamin A and lamin B was not affected by these compounds (Figure 2B). Additionally, a reduction of p16INK4A expression was detected in response to treatment with the JH chemicals (Figure 2B). We also observed the different localization of progerin from that of lamin A upon JH4 treatment (Figure 2C and Supplemental Figure 3C). Moreover, all these JH compounds, and in particular JH4, blocked nuclear deformation (Supplemental Figure 4, A and B). These results indicate that JH4 (and JH13 to some extent) blocked the interaction between progerin and lamin A and alleviated progerin-induced nuclear deformation. However, JH4 did not show any significant effect on the localization pattern of other laminaopathy-related lamin A mutants (2) such as nuclear speckles of D192G (Supplemental Figure 4C). This result also supports the idea that JH4 possesses selective activity on progerin. Next, we examined the effect of JH chemicals on HGPS cells. Consistent with the above results, JH chemicals, in particular JH4, blocked the interaction between progerin and lamin A in these progeroid cells (Figure 2D and Supplemental Figure 4D), without alteration of progerin mRNA expression (Supplemental Figure 4E). In addition, JH chemicals, and especially JH4, ameliorated the frequency of nuclear deformation of HGPS cells (Figure 2, E and F, and Supplemental Figure 4, F and G).

Specific interaction of JH4 and progerin. To determine the direct target of JH chemicals, we monitored their effect on the previously observed progerin-p14ARF binding (14). In this assay, we found that JH chemicals blocked the interaction between progerin and p14ARF (Supplemental Figure 5A), but not between p53 and p14ARF (ref. 25 and Supplemental Figure 5B). In addition, JH4 did not block the interaction between progerin and MEL18/BMI1 that is achieved by the middle region of lamin A (ref. 26 and Supplemental Figure 5, C-E). Considering these facts, the C-terminal region of progerin may be the direct target of JH4 (Supplemental Figure 5F). To verify this hypothesis, we generated biotinylated JH4 (Supplemental Figure 5G; B33: precursor for biotinylation; 07: biotinylated JH4; 08: biotin plus linker). Modified chemicals showed inhibitory activity similar to that of JH4 on binding between lamin A and progerin (Supplemental Figure 5H) as well as on nuclear deformation (Fig-
Figure 2. Ameliorating effect of JH chemicals on nuclear deformation. (A) Specific effect of JH chemicals on the binding of lamin A-progerin but not on that of lamin A-lamin A. GST pull-down assay using lysates from HEK293 cells transfected with GFP-LMNA after incubation with GST-LMNA or GST-progerin under treatment with the indicated chemicals. (B) Dissociation of lamin A and progerin by JH4 in human cells. Dissociation of progerin from lamin A/C was confirmed by IP in HEK293 cells. For this, GFP-progerin–transfected HEK293 cells were incubated with the indicated chemicals (5 μM) for 24 hours. A reduction of p16INK4A was also detected in whole-cell lysate. Actin was used as a loading control. SUP, supernatant. (C) JH4 blocked the colocalization of lamin A and progerin. For this, HEK293 cells were cotransfected with GFP-LMNA and nontagged progerin (NT progerin) for 24 hours and incubated with JH4 for an additional 24 hours. JH4 could abolish the colocalization of lamin A and progerin (red) in the nuclear membrane or in nuclear speckles. DAPI was used for DNA staining. Scale bar: 10 μm. (D) JH4 blocks the interaction of lamin A and progerin in HGPS cells. Treatment with 5 μM JH4, but not FTI-277, for 24 hours blocked the interaction of lamin A and progerin in HGPS cells. Cell lysates were immunoprecipitated with a lamin A–specific Ab (H-102, sc-20880) and immunoblotted with a progerin–specific Ab. Normal cells (9 yr; N9) were used as a negative control for progerin. (E) Effect of JH chemicals on nuclear deformation of HGPS cells. Three kinds of HGPS cells were incubated with the indicated chemicals for 24 hours and fixed for lamin A/C immunofluorescence analysis (green). The same effect was observed in other HGPS cells (Supplemental Figure 4F). Scale bars: 10 μm. (F) Quantification of nuclear abnormalities in HGPS cells. JH chemicals, in particular JH4, ameliorated nuclear deformation of HGPS cells. P values were determined by Student’s t test. PPT, protein pellet.

Figure 8C). These results suggest that JH chemicals, in particular JH4 on oncogene-induced or growth factor–induced senescence (31, 32). Senescence induced by IGF-1 treatment or transfection of oncogenic Ras into WI-38 (normal foreskin fibroblast) was not blocked by JH4 (Supplemental Figure 7, G and H), because oncogene-induced senescence did not induce progerin (Supplemental Figure 7I), nor was induction of p16 blocked by JH4 treatment (Supplemental Figure 7I). In addition, JH4 did not promote cell viability in human cancer cell lines, despite a 72-hour treatment (Supplemental Figure 8A), suggesting that the antisenescent effects of JH4 do not result in the promotion of cancer progression. Instead, JH4 could ameliorate the nuclear deformation in renal cell carcinoma induced by progerin expression (Supplemental Figure 8B), with slight growth suppression at a high concentration (Supplemental Figure 8C). These results suggest that JH chemicals, in particular JH4, selectively block progerin-mediated senescence, without growth promotion of progerin-negative cells.

Restoration of aging-related gene expression profile by JH4 treatment. To gain further insight into the biological effects of JH4, we investigated its impact on gene expression profiles. For this purpose, we hybridized oligonucleotide microarrays with RNA samples from HGPS-1, JH4-treated HGPS, young (9-year-old; N9), and old (81-year-old; N81) fibroblasts. Among 14,400 genes
present in the array, 1,921 showed differential expression (cutoff of 2-fold; Figure 5A and Supplemental Figure 9A). After elimination of nonsignificant changes resulting from the comparison between HGPS and young cells (Supplemental Figure 9A and Supplemental Table 1), we classified differential genes in an HGPS unique gene set (HUG), an aging unique gene set (AUG), and an HGPS/aging-related gene set (HARG) (Figure 6A, Supplemental Figure 9B, and Supplemental Tables 2 and 3). Next, we defined a set of genes whose expression is restored by JH4 treatment (HARG/JR) (298 genes; Supplemental Figure 9B and Supplemental Table

**Figure 3. Progerin is a direct target of the JH4 chemical.** (A) Effect of biotinylated JH4 on nuclear deformation of HGPS cells. Cells were incubated with the indicated chemicals for 24 hours and fixed for lamin A/C immunofluorescence analysis (green). Scale bars: 10 μm. (B) Localization of JH4 in progerin-transfected HEK293 cells. JH4-biotin (07), but not biotin alone (08), was stained in the nuclear membrane by streptavidin-HRP in progerin-transfected cells. Prg, progerin. Original magnification, ×200. (C) JH4 locates in the nuclear membrane. After treatment with biotinylated JH4 for 48 hours, cells were fixed and stained with streptavidin-FITC. Green signal was detected only in JH4-treated HGPS. Scale bars: 10 μm. (D) Specific interaction of JH4 and progerin. Streptavidin-biotin-binding assay using lysates from HGPS cells incubated with biotinylated JH4 or biotin. After incubation with streptavidin-coated magnetic beads, the biotinylated JH4 with the protein complex was isolated. (E) Direct and specific interaction between JH4-biotin and progerin. Cells incubated with the indicated chemicals were lysed with RIPA and incubated with streptavidin-coated beads. Material precipitated by the biotin-streptavidin-bead complex was analyzed by Western blot analysis. GFP-progerin was precipitated by the biotin-streptavidin complex in JH4-biotin–treated cells. However, other nuclear membrane proteins such as lamin B (LMNB), emerin and GFP–lamin A, and p53 were not associated with JH4-biotin. WCL, whole-cell lysate.
4). To verify these transcriptomic results, we confirmed that the expression of several genes such as *IL33*, *BRCA1*, *BLM*, *RAD51*, *IL6*, *IL8*, and tumor necrosis factor superfamily member 18 (*TNFSF18*) was altered at both the protein and mRNA levels after JH4 treatment (Figure 6, B and C). By contrast, farnesyltransferase inhibitor (FTI) treatment did not revert the expression changes of the HARG/JR genes (Supplemental Figure 9C), indicating that the
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The expression levels of IL33, RAD51, and BRCA1 were reduced in 3 kinds of aged fibroblasts (N81; N72, 72-year-old; and N94, 94-year-old) and HGPS-2 cells compared with the levels detected in fibroblasts from N9 (Supplemental Figure 9D). In contrast, IL6 and IL8 were overexpressed in these cells (Supplemental Figure 9D). This result suggested that alternation of these expression levels would result from aging. To investigate the biological significance of these transcriptional alter-

Figure 5. Antisenescence effect of JH chemicals on normal aged cells. (A) Nuclear deformation of aged fibroblasts was ameliorated by JH4. Normal cells obtained from 9-year-old (N9) and 81-year-old (N81) subjects were incubated with JH4 (5 μM) for 48 hours and stained with anti–lamin A/C Ab (green). DAPI was used for DNA staining. Scale bars: 10 μm. (B) JH4 could induce cell proliferation in aged fibroblasts. After seeding 7.5 × 10^4 cells per well, cells were maintained for a maximum of 6 days with the indicated JH chemicals (5 μM). Cell numbers were calculated every 48 hours. The slow proliferation of cells from N81 compared with that of cells from N9 (Figure 4B) was completely rescued by JH4 treatment. *P = 0.013. The P value was determined by Student’s t test for analysis of statistical significance between 2 groups. (C) JH4 suppressed senescence of N81 fibroblasts. An SA–β-gal assay was performed on cells from N81 after treatment with the indicated chemicals (5 μM) for 48 hours. Original magnification, ×40. (D) JH4 induced H3K9Me3 expression in normal aged cells. Immunofluorescence for H3K9Me3 (green) was performed in N9 and N81 cells under treatment with the indicated chemicals (5 μM) for 48 hours. Scale bars: 10 μm. (E) JH4 suppressed DCR2 and p16<sup>INK4A</sup> in normal human cells. Reverse transcriptase-PCR (RT-PCR) analysis of the indicated genes was performed in normal cell lines (N9, N29, and N81) after treatment with JH4 for 48 hours.
Figure 6. Aging-related gene expression is recovered by JH4 treatment. (A) Heatmap of gene expression profiles showing a difference of at least 2-fold in both cell lines. Colors represent values normalized to the range of intensity for each gene in all conditions. (B) Reduced expression of IL33, BRCA1, BLM, and RAD51 in HGPS cells was reverted by JH4. Conversely, IL6, IL8, and TNFSF18 overexpression was suppressed by JH4 treatment. (C) JH4 could alter HGPS-specific gene expression. RAD51, BRCA1, cyclin B1, and CDC25C protein expression levels were increased by treatment with JH4. (D) Network analysis using cBioportal. Genes in the HARG/JH group were clustered into chromosome separation, DNA replication, DNA repair, and cell-cycle groups. (E) JH4 overcame progerin-induced cell-cycle arrest. Cell-cycle arrest at the G2/M phase by progerin transfection was released by JH4 treatment (5 μM, for 24 hours).
Figure 7. JH4 prevents progeroid phenotypes in LmnaG609G/G609G mice. (A) Representative photographs of 8-week-old LmnaG609G/G609G female mice (left; male, right) treated with JH4 (10 mg/kg twice per week). Mice were injected 8 times (from 4 weeks of age). (B) Increase in BW of LmnaG609G/G609G mice following JH4 injection. P values were determined by Student’s t test. (C) Increase in muscle strength following JH4 injection. Grip strength was measured for LmnaG609G/G609G, Lmna+/+; and Lmna−/− male mice by pulling the spring balance after treatment with JH4 or vehicle alone. P value was determined by Student’s t test. (D) Growth morphology of thymus and spleen from 9-week-old LmnaG609G/G609G mice treated with vehicle or JH4. An obvious enlargement of both organs after JH4 treatment was observed. Compared with vehicle-injected mice, the size of several organs was gradually increased by treatment time. Numbers above each bar indicate the fold increase at each time point (see also Supplemental Figure 14). (F) JH4 injection promoted the cell cycle of splenocytes obtained from 7-week-old mice (injected 6 times). Cell-cycle promotion was observed in JH4-treated cells. (G) Rescue of cell and tissue defects. Foot pad skin was thickened and dermal connective tissue was enriched in JH4-treated animals (upper panels). Kidney tissue was well organized after JH4 treatment. Tissues were obtained from 18-week-old (vehicle) and 22-week-old (JH4) mice. Scale bar: 80 μm, original magnification ×100. (H) Increase in cell density and reduction of deformed cell nuclei in JH4-treated heart tissue obtained from the same mice above. On the basis of the histology, nuclear morphology (right) and cell density (left) were counted for 3 different mice and 5 different regions per mouse by 3 researchers. P values were determined by Student’s t test. (I) Representative images of heart muscle. Arrows indicate deformed nuclei. Scale bar: 10 μm. (J) JH4 suppressed progerin and induced RAD51 and phosphorylated CDC2 (p-CDC2). Western blot analysis was performed using extracts from liver and kidney tissue obtained from 18-week-old sibling mice.

In all examined tissues, JH4 treatment increased cell density and reduced the number of deformed nuclei. For example, the reduction in cell numbers in the same region or in tubes and
Figure 8. Lifespan extension in progerin-expressed mice. (A) Representative photograph of 40-week-old Lmna<sup>G609G/G609G</sup> control or JH4-treated mice. (B) BW of 40-week-old Lmna<sup>G609G/G609G</sup> control and JH4-treated mice. (C) In vivo effect of JH4 on binding between progerin and lamin A/C. Lmna<sup>G609G/G609G</sup> mice that received a single injection of JH4 (10 mg/kg) were sacrificed for IP analysis of liver and kidney. After 6 hours, JH4 could disrupt the interaction of progerin and lamin A. After 24 hours, dissociation of progerin and lamin A was detected in kidney. Extracted proteins were immunoprecipitated with progerin Ab, and the precipitated proteins were subjected to Western blotting with anti–lamin A/C or anti-progerin Ab. (D) Detection of JH4 in liver. Biotin–JH4 was injected into Lmna<sup>G609G/G609G</sup> mice, and liver lysates were reacted with streptavidin beads. Precipitated proteins were analyzed by Western blotting. (E) Kaplan–Meier survival plots for Lmna<sup>G609G/G609G</sup> mice treated with JH4 (n = 13) or with vehicle alone (n = 11). (F) Kaplan–Meier survival plots for Lmna<sup>G609G/G609G</sup> mice treated with vehicle (n = 6) or JH4 (n = 10). (G) Extension of lifespan by high-dose injection of JH4. A dose of 20 mg/kg JH4 could extend lifespan by as much as 26 weeks. (B and E–G) P values were determined by Student’s t test.

Specific effect of JH4 on progerin-expressing mice. Because Lmna<sup>G609G/G609G</sup> progerin heterozygous mice also exhibit progeroid features, we checked the effect of JH4 on these animals and found that injection of JH4 (twice/week) over a 20-week period also ameliorated a number of aging phenotypes present in these mice, such as loss of BW (Figure 8, A and B, and Supplemental Figure 14A) and dermal and heart muscle alterations (Supplemental Figure 14B). Moreover, treatment of Lmna<sup>G609G/G609G</sup> mice with JH4 could result in recovery of their BW to that of normal mice (Figure 8B). To investigate whether the in vivo antiprogerin effects of JH4 are due to the inhibition of progerin–lamin A binding or to an unknown side effect of this compound, we decided to test JH4 in a progerin-independent progeria model, namely the zinc metalloendopeptidase STE24-deficient mouse (Zmpste24<sup>-/-</sup>) (38), in which the progeroid phenotype is caused by prelamin A rather than by progerin accumulation. Despite the nearly identical progeroid phenotype of Zmpste24<sup>-/-</sup> and Lmna<sup>G609G/G609G</sup> mice, JH4 did not produce any noticeable change of growth in Zmpste24<sup>-/-</sup> mice (Supplemental Figure 14C), demonstrating that the antiaccelerated aging activity of JH4 is directly related to its inhibition of progerin’s deleterious effects. Indeed, the interaction between progerin and lamin A/C in tissues was disrupted by a single JH4 injection (Figure 8C), following its metabolic trap. Disruption of progerin–lamin A/C binding by JH4 in liver at 6 hours was observed after 24 hours in kidney. We were able to recover the progerin–binding biotin–JH4 (labeled “07” in Figure 8D) in liver 6 hours after injection, indicating, as we expected, that JH4 could work in an in vivo system.

JH4 extends lifespan in the progerin mouse model. Next, we assessed the lifespan of Lmna<sup>G609G/G609G</sup> progeroid mice after JH4 injection. As shown in Figure 8E, the lifespan of JH4-treated progeroid mice was extended by more than 4 weeks compared with that of vehicle-treated mice. Although JH4 treatment did not fully overcome the short lifespan of these animals, considering the low concentration (10 mg/kg) and frequency (2 times/week) of administration, it appears to have a promising effect. In fact, JH4 also significantly extended the lifespan of Lmna<sup>G609G/G609G</sup> heterozygous mice (Figure 8F). Since biological effect of JH4 was dose-dependent, we injected 20 mg/kg JH4 into Lmna<sup>G609G/G609G</sup> progeroid mice. From this experiment, we found that 20 mg/kg JH4 could extend lifespan by as much as 26 weeks (Figure 8G). These results suggest that if JH4 can be retained at a higher concentration, lifespan could be more dramatically extended and that JH4 would be a strong therapeutic drug candidate for the treatment of HGPS.

Discussion

The aging process is complicated, and a full understanding of the molecular mechanisms controlling it is not likely to be realized in the near future. However, some basic clues are known from studying human progeria syndromes such as HGPS (39–41). Although HGPS is caused by progerin, a product of a point mutation in the LMNA gene, progerin is also expressed in normal aged cells as well as in cancers (14). Thus, understanding the pathological function of progerin is useful for the development of a therapeutic strategy to treat HGPS as well as for the prevention of aging.

In fact, over the past decade, the farnesylation of progerin has been targeted for drug development to treat HGPS. (24, 42). Because the farnesylated domain of progerin rigidly anchors it to the nuclear membrane, it may induce nuclear deformation. Blocking farnesylation by FTIs in HGPS cells restores nuclear morphology, cell proliferation, and heterochromatin organization. Moreover, treatment with FTIs in HGPS-like mouse models improves lifespan, growth, and BW and relieves premature aging symptoms such as bone defects (23). In particular, FTIs can extend the lifespan of Zmpste24<sup>-/-</sup> mice (24). However, a clinical trial of FTI did not produce promising results (43), indicating that other targets should be explored.

In this study, we found that the most important target of progerin is lamin A/C. Indeed, progerin shows strong binding affinity for lamin A/C but not lamin B (Figure 1E). On this basis, we found new chemicals that can block the interaction between progerin and lamin A/C through direct interaction with progerin (Figure 2B and Figure 3E). Since the chemical JH4 can bind selectively to progerin, there would be very minimal, nonspecific side effects. In our study, JH4 did not show an effect on the progerin-independent progeria model (Zmpste24<sup>-/-</sup> mice; Supplemental Figure 14C) or on mouse and monkey cell lines (data not shown). These results indicate that JH4 has potential use as a progerin-inhibitory drug.

Although JH4 did not fully overcome the short lifespan of the Lmna<sup>G609G/G609G</sup> mouse model, given the low dose and infrequent injections of the compound, an optimized treatment strategy using JH4 could offer an effective therapy. Indeed, the same scheduled injection produced more obvious effects in Lmna<sup>G609G/G609G</sup> mice (Figure 8), and JH4 appeared to be excreted within 24 hours.

In summary, in this study, we have identified 3 compounds, JH1, JH4, and JH13, that efficiently block progerin–lamin A/C
binding and reverse or alleviate different senescence features characteristic of HGPS cells, including growth arrest, SA-β-gal accumulation, and nuclear aberrations. Moreover, we observed similarly favorable effects of JH chemicals on normal aged fibroblasts, which also express progerin. Indeed, JH4 suppressed SA-β-gal activity and increased the proliferation of these cells. Moreover, many genes altered by JH4 treatment were common between HGPS and aging cells. The fact that this compound also rescued several progeroid features of Lmna
G609G/G609G mice and extended the lifespan of this HGPS mouse model represents a proof of principle for the in vivo relevance of blocking progerin– lamin A/C interaction as a new treatment strategy for HGPS and, eventually, for age-associated alterations involving nuclear envelope abnormalities.

Methods

Animal experiments. Progerin-heterozygous Lmna
G609G/G609G mice were provided by Carlos López-Otin (Universidad de Oviedo, Asturias, Oviedo, Spain). Timed matings of heterozygous Lmna
G609G/G609G and homozygous Lmna
G609G/G609G males and females were conducted, and chemical intraperitoneally injected for indicated weeks or months (Figure 8, A–G). All mice were maintained on outbred C57BL/J backgrounds.

Histology. Following sacrifice at the end of the injection regimen, the indicated organs (foot pad, kidney, and heart) were isolated in PBS and fixed in 10% formaldehyde in PBS at 4°C for 6 to 12 hours. Tissue was dehydrated using ethanol (50%, 70%, 90%, 3 × 100%; 5 minutes each) and paraffin embedded for sectioning. Sections (5-μm-thick) were used for H&E staining.

Chemical screening and chemical synthesis. For chemical screening, an ELISA was established by modifying a previously described platform (44). Details on the synthetic methods used are provided in the Supplemental material.

Cell culture and reagents. Human fibroblast cells were obtained from the Coriell Cell Repositories. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid from the Coriell Cell Repositories. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid. Human cancer cell lines (HCT116, A549, and HEK293), obtained from ATCC, were maintained in liquid.


