Supplementary Materials

Progerin and telomere dysfunction collaborate to trigger cellular senescence

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Figure S1: a. Live cell imaging of normal primary fibroblasts (AG06299) transfected with the progerin-splicing reporter. Arrow: an example of a DsRed-only cell; Arrowhead: an example of a double-positive cell. b. Immunoprecipitation followed by western blotting analysis with an anti-GFP antibody in whole cell lysates from an un-transfected fibroblast line (-), and a reporter-transfected cell lines (+). GAPDH was used as a loading control.
Figure S2: The percentage of fibroblast cells that utilize the cryptic LMNA splice site increases with cell passage number, but not with age of the skin biopsy donor.
Figure S3: Validation of progerin and lamin A specific primers. a RT-PCR analysis of normal and HGPS fibroblasts using primers detecting either lamin A or progerin. N1: HGADFN168 (normal); N2: HGADFN090 (normal); H1: HGADFN003 (HGPS); H2: HGADFN167 (HGPS). Final PCR products after 40 amplification cycles were shown, so this is not a quantitative analysis. b PCR analysis using purified progerin or lamin A cDNA as template. c Amplification efficiency of progerin and lamin A primers on purified cDNA. The efficiencies are 93.3% and 61.3% for progerin and lamin A primers, respectively.
Figure S4: Box plot presentation of the significant elongation of telomere length in hTERT-immortalized fibroblast cells AG09838 in comparison to untreated primary parent cells (p < 0.0001).
Figure S5: DNA sequencing confirms the heterozygous point mutation (K902N, C=>G) in the hTERT gene in cell lines JH-1 and JH-2.
Figure S6: Immunostaining of a normal fibroblast cell line AG08470 with anti-lamin A/C and anti-progerin antibody.
Figure S7: Immunofluorescence staining with an anti-progerin antibody on HeLa cells transfected with GFP-lamin A or GFP-progerin. The pictures were taken with the sample exposure time. The progerin antibody only stains the cells with GFP-progerin signals, and does not label the GFP-lamin A expressing cells.
Figure S8: Images of lamin A/C cytoplasmic stainings under over-exposure condition. We observed some co-localization of signals from progerin and lamin A/C antibodies. Scale bar: 20µm.
Table S1: Gene lists as described in Figure 7A. Quantitative RT-PCR assays were carried out to validate these changes: out of the 8 randomly-selected genes, 5 were validated (62.5%).

Table S2: The 82 overlapping genes from Figure 7B that exhibit significant changes in alternative splicing as cells senesce.

Table S3: The cell lines used in this study.