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Exacerbated atherosclerosis in progeria is prevented by progerin elimination in vascular smooth muscle cells but not endothelial cells

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Hutchinson–Gilford progeria syndrome (HGPS) is a rare disease caused by the expression of progerin, a mutant protein that accelerates aging and precipitates death. Given that atherosclerosis complications are the main cause of death in progeria, here, we investigated whether progerin-induced atherosclerosis is prevented in *HGPSrev-Cdh5-CreERT2* and *HGPSrev-SM22α-Cre* mice with progerin suppression in endothelial cells (ECs) and vascular smooth muscle cells (VSMCs), respectively. *HGPSrev-Cdh5-CreERT2* mice were undistinguishable from *HGPSrev* mice with ubiquitous progerin expression, in contrast with the ameliorated progeroid phenotype of *HGPSrev-SM22α-Cre* mice. To study atherosclerosis, we generated atheroprone mouse models by overexpressing a PCSK9 gain-of-function mutant. While *HGPSrev-Cdh5-CreERT2* and *HGPSrev* mice developed a similar level of excessive atherosclerosis, plaque development in *HGPSrev-SM22α-Cre* mice was reduced to wild-type levels. Our studies demonstrate that progerin suppression in VSMCs, but not in ECs, prevents exacerbated atherosclerosis in progeroid mice.

Hutchinson–Gilford progeria syndrome | atherosclerosis | vascular smooth muscle cells | endothelial cells

Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a heterozygous mutation in the *LMNA* gene that provokes wide expression of progerin, a mutant version of the nuclear protein lamin A that accelerates aging and precipitates death (average lifespan: 14.6 y). Patients develop severe atherosclerosis, which in most cases leads to fatal myocardial infarction, stroke, or heart failure (1).

Gene editing has emerged as a promising approach to reducing progerin expression (2–5). To optimize gene therapy in HGPS, it is important to identify the appropriate time window for intervention and the cell types in which progerin elimination yields more benefit. To tackle these questions, we previously generated progeroid *HGPSrev* mice which ubiquitously express progerin, lack lamin A, and allow time- and cell type–specific progerin suppression and lamin A restoration (6) (*SI Appendix*). Here, we investigated whether HGPS-associated atherosclerosis is prevented upon progerin suppression and lamin A restoration in endothelial cells (ECs) or vascular smooth muscle cells (VSMCs), key vascular cell types in atherogenesis.

Results and Discussion

We generated *HGPSrev-Cdh5-CreERT2* mice with tamoxifen-inducible, EC-specific progerin elimination and lamin A restoration. We treated wild-type (*WT*), *HGPSrev*, and *HGPSrev-Cdh5-CreERT2* mice with tamoxifen at 1.5 mo of age, long before the first HGPS-like signs in *HGPSrev* mice (6), and analyzed progerin expression in 12-mo-old mice. Immunofluorescence assays confirmed efficient progerin elimination in luminal ECs in *HGPSrev-Cdh5-CreERT2* aorta (Fig. 1*A*) and EC-specific progerin suppression in the *HGPSrev-Cdh5-CreERT2* aorta, heart, liver, and kidney (Fig. 1*B*). RT-qPCR analysis confirmed progerin suppression and lamin A restoration in ECs but not macrophages from *HGPSrev-Cdh5-CreERT2* hearts (Fig. 1*C*).

HGPSrev-Cdh5-CreERT2 mice had the characteristic alterations of *HGPSrev* mice with ubiquitous progerin expression, including body weight loss, premature death, aortic VSMC loss, and exaggerated medial collagen deposition (Fig. 1 *D* and *E*). *HGPSrev* mice showed increased leukocyte accumulation in the aortic intima compared with *WT* controls, which was not significantly reduced in *HGPSrev-Cdh5-CreERT2* mice (Fig. 1*F*). We studied the effects of progerin suppression and lamin A restoration in ECs after inducing atherosclerosis by intravenous injection of an adeno-associated virus encoding the mouse PCSK9 gain-of-function

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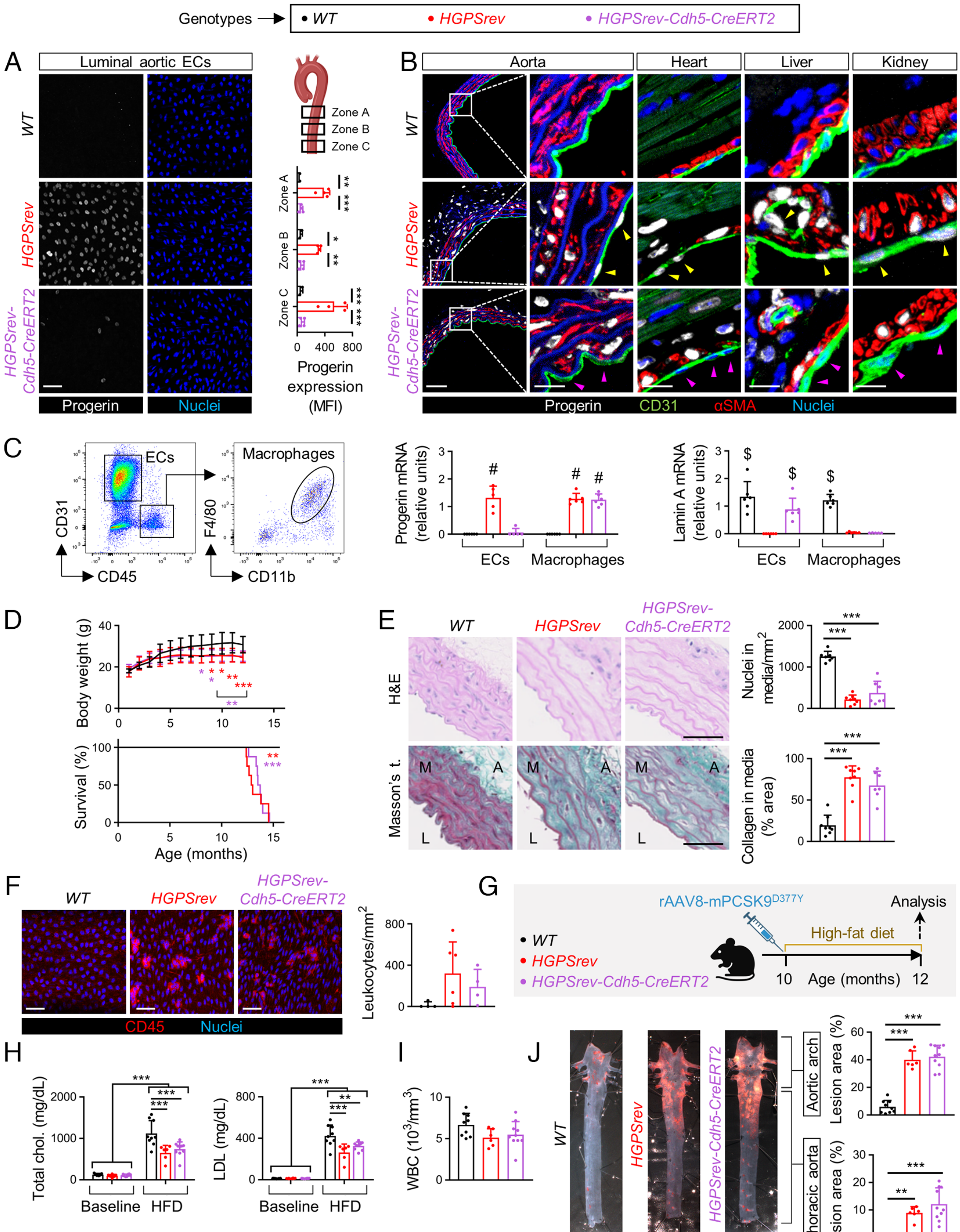

Fig. 1. Endothelium-specific progerin suppression and lamin A restoration do not prevent body weight reduction, lifespan shortening, and excessive atherosclerosis. (*A*) En face immunofluorescence in three different zones of thoracic aortas showing progerin (white) and nuclei (blue) in luminal ECs (*WT*, n = 2; *HGPSrev* and *HGPSrev-Cdh5-CreERT2*, n = 4). (Bar, 50 μm.) MFI, mean fluorescence intensity. (*B*) Immunofluorescence on tissue cross-sections showing progerin (white), CD31 (green), αSMA (red), and nuclei (blue) (n = 4). [Bar in the aorta (*Left*), 50 μm; bar in the zoomed aorta and heart, liver, and kidney, 10 μm.] Yellow and pink arrowheads indicate progerin-positive and progerin-negative ECs, respectively. (*C*) *Left:* Gating strategy for the isolation of mouse cardiac ECs and macrophages by cell sorting. *Right:* RT-qPCR analysis of progerin and lamin A mRNA expression in cardiac ECs and macrophages (n = 5 to 6). $^{\#}P < 0.001$ vs. ECs from *WT* and *HGPSrev-Cdh5-CreERT2* mice and macrophages from *WT* mice. $^{\$}P < 0.001$ vs. ECs from *HGPSrev* mice and macrophages from *HGPSrev* and *HGPSrev-Cdh5-CreERT2* mice. (*D*) *Top*: Body weight (*WT*, n = 13; *HGPSrev* and *HGPSrev-Cdh5-CreERT2*, n = 8). *Bottom*: Kaplan–Meier survival curves (*WT* n = 5; *HGPSrev* and *HGPSrev-Cdh5-CreERT2*, n = 8). Red and purple asterisks indicate significant differences between *WT* and *HGPSrev* or *HGPSrev-Cdh5-CreERT2* mice, respectively. (*E*) Hematoxylin–eosin (H&E) and Masson's trichrome (Masson's t.) staining of aortic arch sections (n = 7 to 8). (Bars, 50 μm.) L, lumen; M, media; A, adventitia. (*F*) En face immunofluorescence of the luminal surface of thoracic aortas showing CD45 (red) and nuclei (blue) (*WT*, n = 4; *HGPSrev*, n = 6; *HGPSrev-Cdh5-CreERT2*, n = 4). (Bars, 50 μm.) (*G*) Protocol for atherosclerosis studies. (*H*) Total cholesterol (chol.) and LDL serum levels (n = 6 to 14). (*I*) Circulating white blood cells (WBC) (n = 6 to 10). (*J*) En face oil red O staining of aortas (n = 6 to 10). (Bars, 5 mm.)

mutant PCSK9^{D377Y} (rAAV8-mPCSK9^{D377Y}) followed by 2 mo of high-fat diet (HFD) (7) (Fig. 1*G*). Total cholesterol and low-density lipoprotein (LDL) serum concentrations were markedly higher in all genotypes post-HFD compared with baseline (Fig. 1*H*), and the concentration of circulating white blood cells at end point was similar among genotypes (Fig. 1*I*). Consistent with our previous studies in

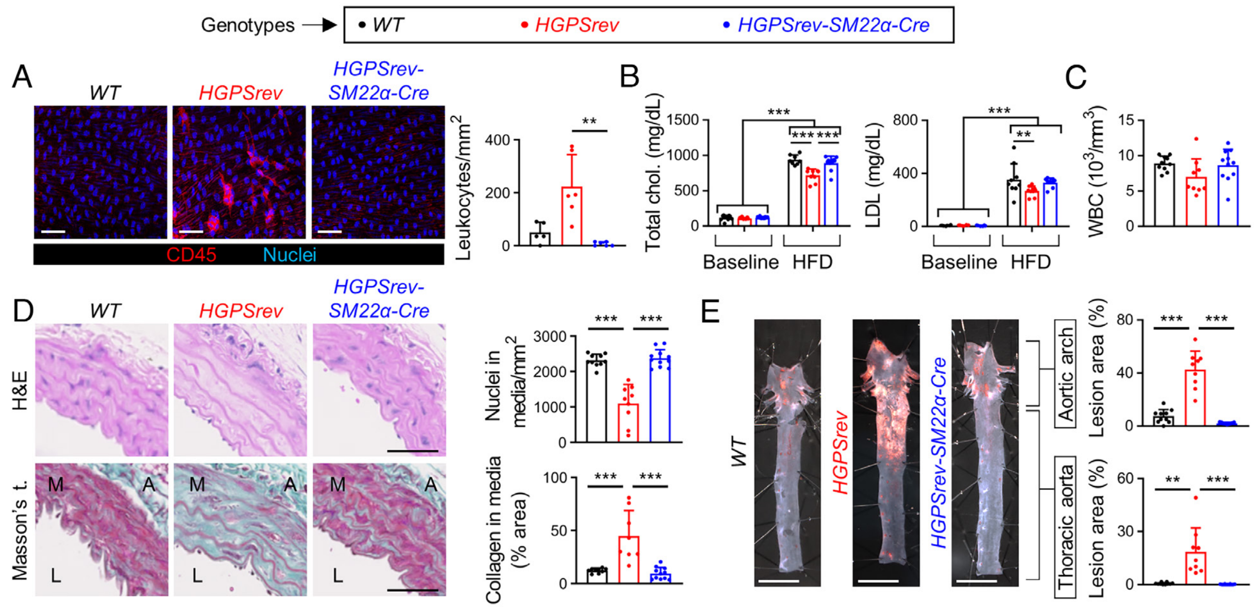

Fig. 2. Progerin elimination and lamin A restoration in VSMCs prevent excessive atherosclerosis. (*A*) En face immunofluorescence of the luminal surface of thoracic aortas showing CD45 (red) and nuclei (blue) (*WT*, n = 5; *HGPSrev* and *HGPSrev-SM22α-Cre*, n = 6). (Bars, 50 μm.) (*B*) Total cholesterol (chol.) and LDL serum levels (n = 8 to 11). (*C*) Circulating white blood cells (WBC) (n = 9 to 11). (*D*) Hematoxylin–eosin (H&E) and Masson's trichrome (Masson's t.) staining of aortic arch sections (n = 8 to 11). (Bars, 50 μm.) L, lumen; M, media; A, adventitia. (*E*) En face oil red O staining of aortas (n = 9 to 11). (Bars, 5 mm.)

other atherosclerosis-susceptible HGPS mouse models with ubiquitous progerin expression (8, 9), aortic atherosclerosis burden was higher in *HGPSrev* mice than in *WT* controls, but this excess of atherosclerosis also occurred upon EC-specific progerin suppression and lamin A restoration (Fig. 1*J*).

We previously demonstrated that *HGPSrev-SM22α-Cre* mice show efficient progerin suppression and lamin A restoration in VSMCs and cardiomyocytes, which prevents HGPS-associated VSMC loss, vascular fibrosis, and premature death (6). *HGPSrev-SM22α-Cre* mice showed reduced leukocyte accumulation in the aortic intima compared with *HGPSrev* mice with ubiquitous progerin expression (Fig. 2*A*). PCSK9^{D377Y} overexpression and HFD (same protocol as Fig. 1*G*) increased total cholesterol and LDL serum concentrations in all genotypes, which showed similar concentrations of circulating white blood cells at end point (Fig. 2 *B* and *C*). Atheroprone *HGPSrev-SM22α-Cre* mice did not show aortic VSMC loss or medial fibrosis (Fig. 2*D*), and their atherosclerosis burden was indistinguishable from that in *WT* mice (Fig. 2*E*).

Although further work is needed to provide mechanistic insights, our studies in *HGPSrev* mice show that progerin suppression and lamin A restoration in VSMCs, but not in ECs, prevent HGPS-associated excessive atherosclerosis, the main driver of premature death in HGPS patients, suggesting that selective VSMC targeting in gene-editing therapies to correct the HGPS-instigating mutation may yield significant therapeutic benefit. Such a strategy would likely require lower doses of gene-editing reagents than those needed for systemic progerin suppression, which may increase opportunities for clinical applications.

Materials and Methods

HGPSrev-Cdh5-CreERT2 mice were generated by crossing *HGPSrev* (6) and *Tg(Cdh5-cre/ERT2)1Rha* mice (MGI ID 3848982). *HGPSrev-SM22α-Cre* mice (6) and PCSK9^{D377Y}-induced atherosclerosis (7) were previously described. Progerin levels were quantified by immunofluorescence and RT-qPCR. Cellular content and collagen accumulation in aorta were analyzed by hematoxylin–eosin and Masson's trichrome staining, respectively. Aortic atherosclerosis was quantified by en face oil red O staining. See details in *SI Appendix*.

Data, Materials, and Software Availability. All study data are included in the article and/or *SI Appendix*.

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