Functional Rescue of Elastin Insufficiency in Mice by the Human Elastin Gene
Implications for Mouse Models of Human Disease

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Abstract—Diseases linked to the elastin gene arise from loss-of-function mutations leading to protein insufficiency (supravalvular aortic stenosis) or from missense mutations that alter the properties of the elastin protein (dominant cutis laxa). Modeling these diseases in mice is problematic because of structural differences between the human and mouse genes. To address this problem, we developed a humanized elastin mouse with elastin production being controlled by the human elastin gene in a bacterial artificial chromosome. The temporal and spatial expression pattern of the human transgene mirrors the endogenous murine gene, and the human gene accurately recapitulates the alternative-splicing pattern found in humans. Human elastin protein interacts with mouse elastin to form functional elastic fibers and when expressed in the elastin haploinsufficient background reverses the hypertension and cardiovascular changes associated with that phenotype. Elastin from the human transgene also rescues the perinatal lethality associated with the null phenotype. The results of this study confirm that reestablishing normal elastin levels is a logical objective for treating diseases of elastin insufficiency such as supravalvular aortic stenosis. This study also illustrates how differences in gene structure and alternative splicing present unique problems for modeling human diseases in mice. (Circ Res. 2007;101:523-531.)

Key Words: elastin ■ supravalvular aortic stenosis ■ vascular disease ■ transgenic mice

Mutations within the elastin gene lead to several elastinopathies in humans that affect large blood vessels, the skin, and the lung. For example, loss-of-function mutations that produce haploinsufficiency have been linked to supravalvular aortic stenosis (SVAS-MIM185500), a congenital narrowing of the ascending aorta and other vessels. SVAS can occur sporadically or as a familial condition with autosomal-dominant inheritance. More than 50 different mutations have been described that lead to isolated SVAS. SVAS is also a component of Williams-Beuren syndrome (WBS-MIM194050), a frequent heterozygous deletion of a ~1.6 Mb segment at chromosome 7q11.23 that includes the elastin gene.

In contrast to the loss-of-function mutations typical of SVAS, evidence suggests that autosomal dominant cutis laxa (ADCL-MIM123700) occurs through a dominant-negative mechanism. ADCL is characterized by lax skin with other internal organ involvement. Most elastin mutations associated with this disease are single nucleotide deletions near the 3' end of the gene resulting in missense sequence that alters the character of a biologically important domain at the end of the tropoelastin molecule. ELN has also been suggested to be a susceptibility gene for hypertension, emphysema, and intracranial aneurysms. ELN encodes a protein made up of alternating hydrophobic and crosslinking domains. This repeating arrangement reflects the exon structure of the gene, with each type of domain encoded by distinct exons. Alignment of human and mouse elastin cDNA sequences shows 64.5% identity at the nucleotide level and 64.1% identity and 72.6% similarity at the amino acid level. This is below the average identity of 85% at the nucleotide level and 78.5% at the amino acid level for human and mouse cDNAs. There are also major differences in the exon content in human and mouse elastin genes. In most mammalian species, the elastin gene is composed of 36 exons distributed throughout approximately 40 kbp of genomic DNA. Rat and mouse Eln have 37 exons attributable to an additional short exon inserted after exon 4. The human ELN gene, however, has only 34 exons attributable to the sequential loss of 2 exons (34 and 35) during primate evolution. In addition, although still contained within the human gene, exon 22 is rarely included in the elastin gene transcript. It is unclear what, if any, selective advantage is conferred on the protein by the loss of 2 exons and the silencing of a third in primate lineages, but these differences in gene structure, together with divergent amino acid sequences between elastin in primates and other species, has important implications for the use of animal models to study diseases associated with mutations in this important human gene.

This report describes a humanized elastin mouse with elastin production being controlled by the human elastin gene...
in a bacterial artificial chromosome (BAC). Elastin from the human transgene is able to reverse the cardiovascular phenotype associated with elastin haploinsufficiency and rescues the lethality of the null phenotype. Importantly, the human BAC elastin transgene retains the human alternative splicing pattern, which makes the human BAC mouse a suitable model to study how ELN mutations lead to human disease. This study also illustrates how differences in gene structure and alternative splicing present unique problems for modeling human diseases in mice and the need for caution in extrapolating information from mouse models to human disease.

Materials and Methods

BAC DNA Purification and Generation of Transgenic Mice

A human BAC clone (CTB-51J22) containing the complete human elastin gene was obtained from Research Genetics. BAC circular DNA was isolated using a Large-construct kit (Qiagen). After the last ethanol precipitation step, DNA was dissolved in injection buffer (10 mmol/L Tris-HCl, pH 7.4, 10 mmol/L NaCl, 0.25 mmol/L EDTA) and then dialyzed on a floating 0.1 μm Millipore membrane filter against the injection buffer.

Isolated BAC DNA was injected at a concentration of 1 ng/mL into fertilized mouse oocytes from C57BL/6 mice, which were implanted into the uterus of pseudopregnant foster mothers. After birth, potential founders were screened for the presence of the transgene using PCR with human elastin specific primers (see online supplemental data, available at http://circres.ahajournals.org, for primer sequences). Animals positive for the transgene were mated to WT (C57Bl/6) animals to stabilize the line. The generation and characterization of Eln−/− mice has been previously reported.22

RNA quantitation, RNase protection assays, histology, protein assays, and vessel physiology assessment were all done using standard techniques, details of which can be found in the expanded Materials and Methods section in the online data supplement.

Results

In this report, hBAC refers to the homozygous human elastin transgene (ELN+/+) expressed from a BAC and Eln to the endogenous mouse elastin gene. WT refers to nontransgenic mouse wild-type (Eln−/+). Compound phenotypes are indicated by the presence or absence of each allele. For example, hBAC-mWT would be homozygous for both the human transgene and mouse endogenous gene (ELN+/+, Eln+/+), hBAC-mHET would be homozygous for the human transgene and heterozygous for the mouse gene (ELN+/+, Eln−/+), and hBAC-mNULL would be homozygous for the human transgene and null for the mouse gene (ELN−/−, Eln−/−).

Generation of hBAC Mouse

Because the elastin gene in humans is structurally different from the murine ortholog, we developed a humanized elastin mouse with elastin production being controlled by the human elastin gene in a bacterial artificial chromosome. The BAC contains no known genes upstream of ELN and only a fragment of LIMK1 3′ to ELN. Thus, ELN is the only functional gene in the BAC (Figure 1A). Mice from C57Bl/6 oocytes injected with the BAC were screened for incorporation of the transgene by Southern blot analysis and 6 founder lines were identified (Figure 1B), with lines 2 and 4 having the highest copy number. Expression of the human elastin gene was detected in all founder lines except for line 6. All mice were maintained in the C57Bl/6 background.

Transgene expression was evaluated by RNase protection assay of RNA from aorta, lung, heart, and skin taken from animals 4 weeks of age. The human elastin transgene was expressed in all elastin-containing tissues with highest expression levels in the aorta (Figure 1C). The pattern of expression over the developmental time course in all examined tissues was similar to that observed for the endogenous mouse gene (Figure 1D). These findings show that the temporal and tissue-specific expression pattern of the human gene is regulated similarly to the endogenous mouse gene, indicating positional-independent control of expression of the human BAC transgene. mRNA expression levels, however, did not correlate with transgene copy number; founder line 3 had the highest mRNA expression levels with a gene copy number about 4 times lower than line 4 (based on Southern analysis). Line 1 showed the lowest expression values even though its gene copy number was approximately equivalent to line 5 (data not shown).

Elastin From the Human Transgene Associates With Mouse Elastin to Form Functional Elastic Fibers That Reverse Characteristics of Elastin Insufficiency

Immunofluorescence analysis using species-specific elastin antibodies showed that elastin from the hBAC transgene associated with the mouse protein to form elastic fibers in arterial vessel walls (Figure 2A). To determine whether mouse elastic fibers containing human elastin are functionally equivalent to those containing only mouse elastin, hBAC-mWT animals from founder lines 3 and 4 were bred with Eln+/− mice to generate the hBAC-mHet genotype. Levels of insoluble elastin were assessed through quantitation of desmosine, which is a unique crosslinking amino acid whose level is proportional to the amount of insoluble, mature protein. Desmosine values showed a ∼40% increase in insoluble elastin in line 3 (high expresser) transgenic animals, thereby confirming that the human protein undergoes proper crosslinking and contributes to the insoluble elastin pool. As expected from the difference in RNA expression, little change in desmosine levels was detected in line 4 (low expresser) animals (Figure 2B). Past studies show that mice heterozygous for the elastin gene have unusual cardiovascular properties resulting from elastin haploinsufficiency.13,23 The best-characterized changes, also found with variable penetrance in humans with SVAS and Williams-Beuren syndrome,22,24 include hypertension, mild cardiac hypertrophy, and an increased number of smooth muscle layers (elastic lamellae) in the arterial wall. Figures 2C and 2D show that both hypertension and altered lamellar number are partially reversed in line 3 hBAC-mHet animals. Mean arterial blood pressure decreased from ∼125 mm Hg to ∼100 mm Hg—values close to WT levels. The number of smooth muscle cell layers (ie, lamellar units) also decreased from ∼10 found in the Eln+/− animals to ∼8 (WT levels) in the ascending aorta of line 3 hBAC-mHet mice. In contrast, there was only a small diminution in mean blood pressure in the low expressing hBAC-mHet line 4,
The effect of human elastin on the mechanical properties of large vessels is documented in Figure 3. We have previously shown that Eln<sup>-/-</sup> vessels have decreased compliance compared with WT animals attributable to a decrease in the elastin to collagen ratio, which makes vessels stiffer. The vessels in hBAC-mHET mice, however, show mechanical properties intermediate between Eln<sup>-/-</sup> and WT, suggesting that elastin from the human transgene is altering vessel compliance toward normal values by increasing the elastin concentration. These results confirm that human elastin from the hBAC transgene is functional and can augment mouse elastin to reverse elastin insufficiency.

The Human Elastin Transgene Retains the Human Alternative Splicing Pattern

Extensive coding diversity is generated from the single-copy ELN gene by alternate splicing of elastin pre-mRNA. Alternative splicing of human and mouse elastin mRNA was compared using RT-PCR and RNase protection assay. In mRNA from the hBAC gene, DNA sequences from RT-PCR products as well as RNase protection studies found deletion in all transcripts of exons 22 and 26A, minor amounts of exon 23 deleted (Figure 4A), and deletion of exon 32 in 50% of the transcripts (Figure 4B). The mouse, in contrast, showed no splicing in this region of the gene. These results demonstrate that the hBAC transgene retains the elastin gene-splicing pattern normally observed in humans and that both the pattern and extent of exon splicing in the human gene is retained in the mouse.

Human Elastin From the BAC-Transgene Rescues the Null Phenotype

To ascertain whether the human elastin gene could completely substitute for the endogenous murine gene, we introduced the BAC transgene into the homozygous Eln<sup>-/-</sup> knockox background by backcrossing line 3 hBAC-mHET animals (Figure 5A). hBAC-mNULL mice were viable, indicating rescue of the perinatal lethality normally observed with elastin deficiency by the human elastin gene.
No animals of the genotype $ELN^{-/-}$, $Eln^{-/-}$ were recovered, however, indicating that the dosage of the hBAC gene on the homozygous knockout background has a direct effect on survival. Immunofluorescence staining of cultured dermal fibroblasts using species-specific elastin antibodies confirmed the absence of mouse elastin in hBAC-mNull animals (supplemental Figure I).

Rescued hBAC-mNull mice were interbred to establish humanized rescued colonies. Although mice were born at the expected Mendelian ratio, $\approx 15\%$ died prematurely between birth and day 5 and another $\approx 45\%$ died between 3 and 5 months of age (supplemental Figure II). Autopsy revealed significant cardiomegaly, suggesting heart failure as a probable cause of death. Surviving hBAC-mNull mice, however, were fertile with long-term survivability ($>2$ year).

**Phenotypic Differences Between Rescued and Normal Mice**

Arterial smooth muscle cells in the aorta of hBAC-mNULL animals formed concentric layers within the arterial wall (Figure 6A). Thin elastic lamellae were evident between the smooth muscle layers, although they were discontinuous and fragmented when compared with lamellae in WT animals. All hBAC-mNULL mice had significantly thickened ascending aortic walls (Figure 6A), with less integrated elastic lamellae. The wall of the abdominal aorta in hBAC-mNULL animals, in contrast, was similar to WT mice except for thinner lamellae. The decreased vessel wall elastin content suggested by the histology was confirmed through quantification of desmosine. Desmosine levels in the abdominal aorta were one-half WT values and those in the ascending aorta approximately one-third WT levels (Figure 6B). Collagen content as
assessed as nmole hydroxyproline per mg protein was elevated by \(\approx 20\%\) (40.8 ± 4.8 nmol compared with WT values of 33.9 ± 2.8 nmol) in hBAC-mNull ascending aorta relative to WT control. In contrast, collagen levels in the abdominal aorta of hBAC-mNull animals were lower compared with WT (72.0 ± 3.7 nmoles hydroxyproline in hBAC-mNull compared with 98.4 ± 5.9 nmoles in WT).

Comparison of 3-month-old hBAC-mNull and Eln\(^{-/-}\) mice revealed a similar average body weight (≈25 g), with no statistically significant difference (\(P>0.02\)) (supplemental Table I). No statistically significant difference in heart rate could be detected between the 2 genotypes (both were in the range of 760 beats per min measured under mild anesthesia).

Blood pressure measurements showed a marked elevation in systolic, diastolic, and mean blood pressures in the rescued mice (Figure 6C) relative to WT levels. Total heart weight to body weight, LV+septum to body weight, and RV to body weight ratios were elevated (15% and 13%, respectively) in hBAC-Null animals (supplemental Table I). Measurement of plasma renin concentrations showed elevation of active renin in rescued mice (60±12 ng/mL/hr for hBAC-mNULL compared with 12±2 ng/mL/hr for hBAC-mWT), suggesting that renovascular mechanisms are active in maintaining the elevated pressures, as has been demonstrated in Eln\(^{-/-}\) mice.\(^{13}\)

As was the case in the aorta, lungs in hBAC-mNULL animals showed a \(\approx 65\%\) reduction in elastin levels. As a result, the animals manifest a form of congenital emphysema characterized by grossly enlarged thoraces, airspace enlargement, and altered mechanics. The lung phenotype has been described in detail in Shifren et al.\(^{29}\)

### Human BAC Elastin Gene Expression Is \(\approx 60\%\) Lower Than the Endogenous Mouse Gene

To determine whether reduced human elastin levels in the transgenic animals result from decreased expression from the

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**Figure 3.** Pressure-diameter relationships for ascending and abdominal aorta from line 3 transgenic and nontransgenic animals. Mean values±SEM, \(n=4\) to 6 for each vessel type. Significant differences between mHET and hBAC-mHET at each pressure value are indicated by * (\(P<0.05\)) and ** (\(P<0.01\)) based on Student t test.

**Figure 4.** Elastin from the human BAC retains the human splice pattern in the mouse, which is different from the splicing of the endogenous mouse Eln gene. A, Alternative splicing of the human and mouse elastin genes from exons 21 to 36 was assessed by RT-PCR and PCR-product sequencing. The mouse gene shows no splicing in this region, whereas the human gene shows complete deletion of exons 22 and 26a and partial deletion of exons 23 and 32. B, RNase protection assay of total mouse aorta RNA with cRNA probes for human elastin message (exon 28 to 36). Densitometric scanning found that the sum of the 2 bands obtained from RNA lacking exon 32 (Ex 28 to 31 and Ex 33 to 36) is approximately equal to the exon 32 included band (Ex 28 to 36), indicating that exon 32 is spliced out of \(\approx 50\%\) of the mRNA transcripts.

**Figure 5.** Human ELN can rescue the mouse null phenotype. A, PCR-Southern analysis of offspring from a hBAC-mHet cross. Animal #9 has the inactivated knockout allele and no wild-type allele (top panel), which corresponds to the null phenotype. The presence of the BAC human elastin transgene (bottom panel), however, rescues the null phenotype resulting in a viable animal.
human gene or increased turnover of the human mRNA relative to the mouse message, semiquantitative PCR analysis was used to assess elastin mRNA levels in the aorta or smooth muscle cells of transgenic animals. Figure 7A shows that hBAC mRNA levels were decreased relative to expression of the endogenous mouse gene. Densitometric scanning of the signal after 26 cycles of PCR suggest a decrease of approximately 60%. This is in agreement with elastin protein values as assessed by desmosine and explains why elastin replenishment in the Eln<sup>-/-</sup> background does not reach WT levels (eg, see Figure 2B). Figure 7B shows results from RNA turnover studies in vascular smooth muscle cells demonstrating that human elastin mRNA is more stable than the mouse transcript. Thus, differences in elastin mRNA turnover do not contribute to the low mRNA levels measured for human elastin.

**Discussion**

In this report we describe a humanized mouse expressing the human elastin transgene in the form of a BAC. Expression of the human and mouse genes in all founder lines except for line 6 showed a similar pattern in multiple tissues, suggesting appropriate temporal and spatial expression of the human transgene. Interestingly, large differences in the human:mouse mRNA ratio were observed, with the human transgene expressed at significantly lower levels. That the human gene retains appropriate spatiotemporal regulation in the mouse when overall gene expression is greatly reduced suggests that the regulatory elements controlling these processes are distinct. It is possible that the elements controlling tissue- and time-specific expression are conserved in the 2 genes, whereas control of expression intensity is regulated by a nonconserved element(s). A comparison of transcription factor binding sites (identified using TESS and TFSEARCH software) in the promoter region up to 2 kb upstream of the ATG shows similarities and differences between the mouse and human gene, consistent with this possibility. In addition, expression profiling of developing mouse aorta<sup>21</sup> found that several transcription factors with binding sites in the human,
sequence at the C terminus of the protein. The single mutations that modify or delete a functionally important levels in untreated human or mouse cells.

Quantitation of insoluble elastin in lung and large vessels of hBAC-mNull animals show a more severe phenotype than mHET mice treated for 24 hours with the RNA polymerase inhibitor 5,6-dichloro-1-o-ribofuranosylbenzimidazole or carrier alone (0.1% DMSO). Elastin mRNA was assessed by semiquantitative RT-PCR, and values expressed as percentage of mRNA alone (0.1% DMSO). Elastin mRNA was assessed by semiquantitative RT-PCR, and values expressed as percentage of mRNA levels in untreated human or mouse cells.

but not mouse, promoter were not expressed in the mouse aorta (data not shown). Another intriguing possibility is that low expression of the human transgene results from the absence of long-range cis-regulatory elements outside the boundaries of the BAC. Such long-range regulation has recently been documented for the region around ELN on chromosome 7, suggesting that elastin insufficiency might also result from long distance regulatory element mutations distinct from any identified in the gene itself.

Evaluation of alternative splicing of the endogenous mouse gene and human transgene found that the human gene produced a transcript with the appropriate human alternative splice pattern, which was different from splicing of the endogenous mouse gene. A human-appropriate splice pattern is important for modeling elastin diseases, especially those that involve mutations in the 3’ region of the gene. Several mutations associated with ADCL, for example, are single base deletions that lead to missense sequence. All are mutations that modify or delete a functionally important sequence at the C terminus of the protein. The single base deletions that have been identified occur within, or in close proximity to, exons that undergo alternative splicing in humans (particularly exons 30 or 32). Whether the mutation produces a missense sequence with a 3’terminally extended open reading frame depends on the splice pattern. For example, a reported single base deletion in exon 30 results in a frameshift that leads to missense sequence and premature termination in exon 32. If exon 32 is spliced out, which occurs ≈50% of the time in humans and in the transgenic BAC, missense sequence continues into the 3’UTR until a new stop codon is encountered downstream of the normal translation termination site. Splicing out of the exon containing the mutation results in normal protein. Because exons 30 and 32 are not spliced in the mouse, mutations in these exons in human, if generated in mice, may have a different biological effect and may not be relevant for functional analysis.

The structural differences in the coding region of the human and mouse genes, combined with differences in alternative splicing, raised the question of whether the human protein could complement mouse elastin or whether the differences in sequence and structure would interfere with normal elastic fiber assembly when expressed in the mouse background. The issue was addressed directly by showing that expression of the human elastin BAC in the Eln<sup>−/−</sup> background raised total vascular insoluble elastin levels by ≈40% and partially reversed the high blood pressure, vessel wall remodeling, and altered vessel compliance that is characteristic of elastin insufficiency. Furthermore, no change in any of these physiological parameters was detected when the human gene was expressed in the WT background (hBAC-mWT), confirming that the human protein was not acting to disrupt mouse fiber assembly. These results provide evidence that human elastin can combine with the mouse protein to form functional fibers despite compositional differences in the 2 proteins. Reversal of the complex traits associated with elastin insufficiency in the hBAC-mHET mouse also confirms that reestablishing normal elastin levels is a logical objective for treating SVAS.

Rescue of the null phenotype by elastin from the human gene supports the functional studies described above in suggesting that assembly of the human protein into a functional elastic fiber is unaffected by the mouse background. However, although levels of elastin in the hBAC-mNULL animals are sufficient to rescue the lethality associated with absence of elastin, they are significantly below normal levels. Quantitation of insoluble elastin in lung and large vessels of these animals shows protein levels to be around one-third the normal values, which is lower than the ≈50% reduction seen in the mELN<sup>−/−</sup> phenotype. The fact that no animals of the genotype ELN<sup>−/−</sup>, Eln<sup>−/−</sup> were recovered indicates that functional elastin levels cannot drop below the 30% level and still support viability.

As predicted from the extremely low elastin levels, rescued hBAC-mNULL mice show a more severe phenotype than what is observed in Eln<sup>−/−</sup> animals. The walls of elastic vessels in hBAC-mNull animals are thicker, contain less elastin, and are less compliant that those in Eln<sup>−/−</sup> mice. Although systemic blood pressure is high in both genotypes,
hBAC-mNULL animals have higher diastolic pressure, which is consistent with their stiffer vessels. As expected from the elevated blood pressure, hBAC-mNULL mice show cardiac hypertrophy characterized by a ≈15% increase in total heart weight and a ≈20% increase in left ventricular weight. Interestingly, there was a ≈40% increase in right ventricular mass, suggesting significant pulmonary hypertension. These changes in ventricular hypertrophy are higher than what was observed in Eln−/− animals (≈13% increase in left ventricular weight, and ≈25% increase in right ventricular weight),13 consistent with greater cardiac stress in the hBAC-mNULL animals. In previous studies we showed that the hypertension associated with elastin insufficiency was correlated with high active renin levels and not attributable to dysfunction of the renin-angiotensin system in maintaining high blood pressure in both genotypes.

The characteristics of the mice described in this study indicate how the phenotypic traits associated with elastin insufficiency directly correlate with elastin levels, which may help explain the phenotypic variability associated with VSAS in humans. Our results also show that restoring elastin to vascular tissue will reverse the effects of VSAS and restore normal vessel function. Because expression of the elastin transgene follows the normal elastin gene expression pattern, the additional elastin is added to the vessel wall during the fetal and early postnatal periods when elastin production is highest.31,32 It will be interesting to determine whether increasing vessel wall elastin in the adult periods has beneficial effects on vessel function.

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Disclosures
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Large arteries are comprised of vascular smooth muscle cells (SMCs) embedded within a complex, cell-derived extracellular matrix. Collagen and elastic fibers, the major constituents of the vascular matrix, are secreted and assembled by SMCs and confer tensile and elastic properties. In the medial layer of elastic arteries, elastin forms concentric fenestrated lamellar layers that intercalate with alternating rings of SMCs to form functional lamellar units. In the aorta, elastic fibers represent the largest component of the extracellular matrix, contributing up to 50% of aortic dry weight.

A series of elegant reports has demonstrated a critical role for elastin in the regulation of vascular morphogenesis in mice. Elastin (eln)-null mice die shortly after birth because of aortic obstruction by SMC proliferation. Heterozygous mice (eln+/−) are viable but produce ≈50% less elastin mRNA and protein these animals are hypertensive, exhibit thinner elastic rings of SMCs to form functional lamellar units. In the aorta, fenestrated lamellar layers that intercalate with alternating medial layer of elastic arteries, elastin forms concentric constituents of the vascular matrix, are secreted and assembled extracellular matrix. Collagen and elastic fibers, the major wall stress.

In this issue of Circulation Research, Hirano et al report the phenotypic rescue of elastin-deficient mice by generation of a humanized elastin mouse. Using a bacterial artificial chromosome encoding the entire human elastin gene (hBAC), they engineered mice to express functional human elastin (ELN) under the control of its native promoter. Several transgenic founder lines demonstrated at least 1 functional ELN insert and were capable of producing human elastin mRNA. Spatial and temporal expression of the human ELN transgene was similar to endogenous mouse elastin. Moreover, the hBAC mRNA product was appropriately spliced, and the protein was correctly secreted, assembled, and incorporated into mouse elastic fibers.

At first glance, it may seem surprising that human elastin can substitute for mouse elastin, considering the differences in exon splicing and the lower than average amino acid conservation between species. In retrospect, however, Hirano et al might have expected the 2 proteins to be interchangeable, because it is primarily the structure of the elastin protein that is important for function. The alternating hydrophobic and crosslinking domains of elastin are conserved throughout vertebrate evolution, and it is this repetitive domain structure that promotes the self-assembly of elastin into fibrillar structures, provides elastomeric properties, and stabilizes elastin to withstand repeated cycles of extension and recoil.

Transgenic expression of human elastin prevented lethality in the eln-null mouse, although the rescued animals expressed only 30% of normal elastin levels. In accordance with the lower elastin content, these animals exhibited a more severe cardiovascular phenotype than eln+/− mice, evidenced by stiffer vessels, higher blood pressure, and greater cardiac hypertrophy. Introduction of the hBAC into the eln+/− mice increased elastin content by 40%, and this resulted in a decrease in lamellar unit number and arterial pressure to levels measured in eln+/− mice, and partially restored vascular compliance. Taken together, these findings suggest a direct relationship between the amount of elastin produced (mouse and human combined) and the severity of the cardiovascular defect in mice. Thus, these mice might provide an elegant system for teasing apart the different thresholds for elastin expression which lead to specific abnormalities in elastic tissues. Indeed, these authors have also used this model to investigate elastin-dosing effects on lung development and susceptibility to smoke-induced emphysema.

In humans, ELN deficiency has been attributed to genetic diseases (reviewed by Milewicz et al10). However no relationship has been established between elastin protein levels and cardiovascular phenotype, although it likely exists based on the wide spectrum of cardiovascular disease severity in patients with elastin deficiencies. Some people hemizygous for ELN can survive into adulthood with little or no cardiovascular problems, whereas in others, the vascular system is severely compromised even before birth. There are clearly other factors, most likely a combination of genetic and environmental, that influence the severity of elastin-related arteriopathy in humans. Population-specific differences in exon splicing and elastin deposition have recently been identified, and 3 quantitative trait loci affecting elastin production have been mapped in rats. Future research in humans and rodents will focus on identifying these important modifier genes, because they will be crucial to implementing successful genetic therapies.

Although certain physiological parameters were improved in the hBAC-null animals, other key functions of elastin were not examined in the current study. Studies of eln+/− mice have revealed that elastin is required to maintain SMC quiescence and circumferential orientation in vivo. These studies are consistent with in vitro findings that insoluble
elastin maintains SMC quiescence, whereas incompletely assembled or degraded elastin peptides promote cell proliferation.18,19 Although other matrix molecules, such as type I collagen, have been implicated in maintaining SMC quiescence, the studies of elastin-deficient mice3,5,7 suggest that in the immediate perinatal phase of development, elastin is the dominant factor. However, the functional consequence of altered SMC orientation remains unclear, as do the mechanisms by which elastin is able to direct SMC orientation and inhibit proliferation. Preliminary studies by Karnik et al21 have begun to dissect these mechanisms, and it will be interesting to see how future studies define the processes involved.

Another benefit of the humanized mouse model is the potential to investigate aspects of ELN function and pathology that are not evident in the eln−null or eln+/− mice. For example, supravalvular aortic stenosis (Online Mendelian Inheritance in Man no. 185500) is the most common cardiovascular manifestation of ELN haploinsufficiency in humans and frequently requires surgical correction15; however, the eln+/− mouse does not exhibit this phenotype. Hirano et al found that introduction of the hBAC to eln+/− mice leads to thickening of the ascending aorta, which may prove to be better model to study SVAS. Moreover, using the humanized mouse, one can test the function of mutations in ELN that produce less severe phenotypes than haploinsufficiency, for example, mutations that affect elastin durability or susceptibility to proteolytic degradation.

In the current study, Hirano et al report copy number–independent transgene expression coupled with very low expression levels, emphasizing the complexity of ELN regulation and suggesting that the mouse milieu is inadequate to recapitulate normal human gene expression. Whether mouse tissues lack specific transcription factors required to drive ELN expression or whether hBAC expression depends on distal regulatory sequences remains to be elucidated. However, as future studies define the missing regulatory elements, humanized mice expressing clusters of relevant genes, rather than individual genes, are likely to be developed. Establishment of normal ELN expression levels in the mouse will provide the most valuable tool for studying ELN and give the greatest opportunity for the development of strategies to treat diseases involving elastin deficiency.

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