

Bouncing Back From Elastin Deficiency

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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.¹ In the aorta, elastic fibers represent the largest component of the extracellular matrix, contributing up to 50% of aortic dry weight.²

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.³ Heterozygous mice (*eln*^{+/-}) are viable but produce ≈50% less elastin mRNA and protein; these animals are hypertensive, exhibit thinner elastic lamellae, more lamellar units, decreased aortic compliance, and mild cardiac hypertrophy compared with *eln*^{+/+} mice.⁴ Extensive experimental studies have revealed that elevated arterial pressure is an adaptation to maintain cardiac output and tissue perfusion in spite of vessel stiffness,⁵ whereas the increase in lamellar unit number is an adaptation to normalize wall stress.⁶

In this issue of *Circulation Research*, Hirano et al report the phenotypic rescue of elastin-deficient mice by generation of a humanized elastin mouse.⁷ 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,⁸ and it is this repetitive domain structure that promotes the self-assembly of elastin into fibrillar structures,⁹ provides elastomeric properties,^{10,11} and stabilizes elastin to withstand repeated cycles of extension and recoil.¹²

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.¹³

In humans, *ELN* deficiency has been attributed to genetic diseases (reviewed by Milewicz et al¹⁴). 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.¹⁵ 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,¹⁶ and 3 quantitative trait loci affecting elastin production have been mapped in rats.¹⁷ 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.³ These studies are consistent with in vitro findings that insoluble

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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,²⁰ the studies of elastin-deficient mice^{3,5,7} 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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Disclosures

None.

References

1. Wolinsky H, Glagov S. A lamellar unit of aortic medial structure and function in mammals. *Circ Res*. 1967;20:99–111.

2. Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix 4: the elastic fiber. *FASEB J*. 1993;7:1208–1218.
3. Li DY, Brooke B, Davis EC, Mecham RP, Sorensen LK, Boak BB, Eichwald E, Keating MT. Elastin is an essential determinant of arterial morphogenesis. *Nature*. 1998;393:276–280.
4. Li DY, Faury G, Taylor DG, Davis EC, Boyle WA, Mecham RP, Stenzel P, Boak B, Keating MT. Novel arterial pathology in mice and humans hemizygous for elastin. *J Clin Invest*. 1998;102:1783–1787.
5. Faury G, Pezet M, Knutsen RH, Boyle WA, Heximer SP, McLean SE, Minkes RK, Blumer KJ, Kovacs A, Kelly DP, Li DY, Starcher B, Mecham RP. Developmental adaptation of the mouse cardiovascular system to elastin haploinsufficiency. *J Clin Invest*. 2003;112:1419–1428.
6. Wagenseil JE, Nerurkar NL, Knutsen RH, Okamoto RJ, Li DY, Mecham RP. Effects of elastin haploinsufficiency on the mechanical behavior of mouse arteries. *Am J Physiol Heart Circ Physiol*. 2005;289:H1209–H1217.
7. Hirano E, Knutsen RH, Sugitani H, Ciliberto CH, Mecham RP. Functional rescue of elastin insufficiency in mice by the human elastin gene. Implications for mouse models of human disease. *Circ Res*. 2007;101:523–531.
8. Chung MI, Ming M, Stahl RJ, Chan E, Parkinson J, Keeley FW. Sequences and domain structures of mammalian, avian, amphibian and teleost tropoelastins: clues to the evolutionary history of elastins. *Matrix Biol*. 2006;25:492–504.
9. Bellingham CM, Lillie MA, Gosline JM, Wright GM, Starcher BC, Bailey AJ, Woodhouse KA, Keeley FW. Recombinant human elastin polypeptides self-assemble into biomaterials with elastin-like properties. *Biopolymers*. 2003;70:445–455.
10. Urry DW, Parker TM. Mechanics of elastin: molecular mechanism of biological elasticity and its relationship to contraction. *J Muscle Res Cell Motil*. 2002;23:543–559.
11. Miao M, Bellingham CM, Stahl RJ, Sitarz EE, Lane CJ, Keeley FW. Sequence and structure determinants for the self-aggregation of recombinant polypeptides modeled after human elastin. *J Biol Chem*. 2003;278:48553–48562.
12. Mithieux SM, Weiss AS. Elastin. *Adv Protein Chem*. 2005;70:437–461.
13. Shifren A, Durmowicz AG, Knutsen RH, Hirano E, Mecham RP. Elastin protein levels are a vital modifier affecting normal lung development and susceptibility to emphysema. *Am J Physiol Lung Cell Mol Physiol*. 2007;292:L778–L787.
14. Milewicz DM, Urban Z, Boyd C. Genetic disorders of the elastic fiber system. *Matrix Biol*. 2000;19:471–480.
15. Eronen M, Peippo M, Hiippala A, Raatikka M, Arvio M, Johansson R, Kahkonen M. Cardiovascular manifestations in 75 patients with Williams syndrome. *J Med Genet*. 2002;39:554–558.
16. Urban Z, Agapova O, Huchtagowder V, Yang P, Starcher BC, Hernandez MR. Population differences in elastin maturation in optic nerve head tissue and astrocytes. *Invest Ophthalmol Vis Sci*. 2007;48:3209–3215.
17. Gauguier D, Behmoaras J, Argoud K, Wilder SP, Pradines C, Bihoreau MT, Osborne-Pellegrin M, Jacob MP. Chromosomal mapping of quantitative trait loci controlling elastin content in rat aorta. *Hypertension*. 2005;45:460–466.
18. Mochizuki S, Brassart B, Hinek A. Signaling pathways transduced through the elastin receptor facilitate proliferation of arterial smooth muscle cells. *J Biol Chem*. 2002;277:44854–44863.
19. Urban Z, Riazi S, Seidl TL, Katahira J, Smoot LB, Chitayat D, Boyd CD, Hinek A. Connection between elastin haploinsufficiency and increased cell proliferation in patients with supravalvular aortic stenosis and Williams-Beuren syndrome. *Am J Hum Genet*. 2002;71:30–44.
20. Koyama H, Raines EW, Bornfeldt KE, Roberts JM, Ross R. Fibrillar collagen inhibits arterial smooth muscle proliferation through regulation of Cdk2 inhibitors. *Cell*. 1996;87:1069–1078.
21. Karnik SK, Brooke BS, Bayes-Genis A, Sorensen L, Wythe JD, Schwartz RS, Keating MT, Li DY. A critical role for elastin signaling in vascular morphogenesis and disease. *Development*. 2003;130:411–423.

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