INVITED EDITORIAL
Elastic-Fiber Pathologies: Primary Defects in Assembly—and Secondary Disorders in Transport and Delivery

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Introduction

Resilience and elastic recoil are properties conferred on all vertebrate elastic tissues by elastic fibers (Cleary and Gibson 1996; Debelle and Tamburro 1999). These complex extracellular matrix biopolymers, assembled from ⩾15 different proteins or glycoproteins, are composed of two distinct morphological entities. The amorphous component is made up of insoluble elastin, a highly cross-linked and hydrophobic protein assembled from soluble precursor proteins called "tropoelastins." The microfibrillar component of elastic fibers is composed of several glycoproteins, of which the best known are the fibrillins (fibrillin-1 and fibrillin-2). Elastic microfibrils are thought to provide a three-dimensional scaffold for the assembly of elastin during the formation of elastic fibers. This elastogenesis occurs in most elastic tissues during late prenatal and neonatal development. It is insoluble elastin that confers to elastic fibers the property of elastic recoil.

In addition to the mechanical properties of resilience, elastic fibers are unique in several other aspects as well. Once synthesized in early development, elastic fibers undergo very little turnover in normal adult tissues, with the notable exception of the uterus. The elastic fibers deposited in one's aorta as a young child, for example, are the same elastic fibers that one usually will die with. In a variety of elastic-tissue diseases, however, new elastic-fiber synthesis in adult tissue results in the aberrant accumulation of dysfunctional elastic fibers. Examples of such common disorders involving aberrant elastic-fiber assembly include emphysema, hypertension, actinic elastosis, and aortic aneurysms.

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Primary Elastinopathies and Fibrillinopathies

To really understand the contribution of abnormal elastic-fiber synthesis to the pathogenesis of such complex elastic-tissue diseases, several investigators during the last few years have turned to heritable, monogenic diseases of elastic fibers. The first disease phenotype that yielded significant insight into the pathogenesis of elastic fibers was Marfan syndrome (MFS [MIM 154700]). Mutations in *FBN1,* a gene that is on chromosome 15 and that encodes fibrillin-1, were shown to result in the ocular, cardiovascular, and skeletal features so characteristic of MFS (reviewed in Dietz and Pyeritz 1995; Ramirez 1996). This work not only established a genetic basis for understanding MFS but also contributed to the realization that fibrillin-1 is a major component of elastic microfibrils. A related disease, congenital contractural arachnodactyly (MIM 121050), subsequently was shown to be due to mutations in *FBN2,* a second fibrillin gene on chromosome 5 (Putnam et al. 1995).

Mutations in the elastin gene also have been shown to result in perturbations of elastic-fiber assembly. Patients with isolated supravalvular aortic stenosis (SVAS [MIM 185500]) develop this stenotic arterial disorder as a result of null mutations in the elastin gene (Urbán et al., in press). SVAS is also a feature in a complex developmental disorder called "Williams-Beuren syndrome" (MIM 194050) in which patients are hemizygous for the elastin gene (Ewart et al. 1993). In patients with Williams syndrome, hemizygosity arises as a result of a microdeletion of ~1.5 Mb on chromosome 7, involving ⩾15 genes and including the elastin gene (for review, see Francke 1999). Mutations in the elastin gene have also very recently been shown to result in at least some examples of an autosomal dominant form of a heritable skin disorder called "cutis laxa" (adCL [MIM 123700]; Tassabehji et al. 1998; Zhang et al. 1999). Patients with adCL are characterized by redundant and inelastic skin, hernias, upper-airway obstruction, mild dilatation of the aorta and great vessels, and, apart from the presence of pulmonary-artery stenoses in both SVAS and adCL, a phenotype strikingly dissimilar to SVAS. Moreover, the elastin-gene mutations in adCL that have been characterized to date are consistent with a domi-

nant negative mechanism that, through the synthesis of an aberrant tropoelastin, probably interferes with elastin assembly. In patients with SVAS, the disruption of elastic-fiber assembly results from reduced synthesis of normal tropoelastin (Urbán et al., in press). Two different classes of mutations in the tropoelastin gene, in other words, seem to result in two, quite different phenotypes.

The mutations in fibrillin genes result in disorders affecting microfibril assembly and are referred to as "fibrillinopathies." Although no mutations have yet been found in genes encoding other microfibrillar proteins such as MFAPs (microfibril-associated proteins), it's entirely possible and indeed very likely that the fibrillinopathies will define a group of diseases due to mutations in several of the genes encoding microfibrillar proteins.

Elastinopathies are diseases due to mutations in just the elastin gene. Together, both fibrillinopathies and elastinopathies can be considered as primary disorders of elastic fibers and as due to mutations in genes encoding proteins that are an integral part of elastic fibers.

Secondary Elastic-Fiber Pathologies

During the past few years, it has become apparent that mutations in genes that encode proteins indirectly involved in elastic-fiber assembly will also result in an elastic-fiber pathology. Menkes syndrome, for example, is an X-linked disorder of copper transport and is due to a spectrum of mutations in a copper-transporting ATPase gene, ATP7A (for review, see Kodama and Murata 1999). Altered copper transport leads to alterations in the activity of a copper-dependent and elastic fiber–associated lysyl oxidase that, through the formation of desmosine cross-links using lysine residues in adjacent tropoelastin molecules in a growing elastic fiber, is responsible for the formation of insoluble elastin. Consequently, altered elastic-fiber morphology is a characteristic feature of elastic tissues in patients with Menkes syndrome (Pasquali-Ronchetti et al. 1994) and contributes to the complex phenotype in this disorder.

Very recently, Aleksander Hinek and his colleagues have demonstrated impaired elastinogenesis in four different diseases that, like Menkes, arise through altered transport mechanisms important to elastic-fiber assembly. Unlike Menkes, however, these elastic-fiber pathologies arise as a consequence of a transport deficiency in the elastin-binding protein.

The 67-kD elastin-binding protein (EBP) chaperones tropoelastin from intracellular sites of synthesis in the RER, through the golgi and secretory vacuoles, to the cell membrane (Hinek and Rabinovitch 1994). EBP is the enzymatically inactive spliced variant of β-galactosidase (called "S-Gal") and a component of a non-integrin cell-surface receptor that delivers tropoelastin to the microfibrillar scaffold of developing elastic fibers (Privitera et al. 1998). Binding of microfibrillar galactosugars to EBP results in both the release of tropoelastin and the recycling of the EBP back to the intracellular endosomal compartment, for reassociation with newly synthesized tropoelastin (Hinek et al. 1995).

Hurler disease (mucopolysaccaridosis type I, or MPSI [MIM 252800]) is an inherited metabolic storage disorder that is caused by a primary defect in lysosomal α-L-iduronidase. MPSI is characterized by a complex phenotype with skeletal, facial, and CNS abnormalities. This deficiency causes an accumulation of both dermatan sulfate and heparin sulfate, two galactosugar-bearing glycosaminoglycans that could possibly disrupt the chaperone function of the EBP and, in so doing, disrupt elastin assembly. In a recent study of this disorder (Hinek and Wilson 2000), it is clear that the accumulation of dermatan sulfate by cultured skin fibroblasts from patients with Hurler disease does result in both the functional inactivation of the EBP and an inability of these fibroblasts to assemble elastic fibers. Interestingly, part of the MPSI phenotypic spectrum is an obstructive coronary arteriopathy very similar to the elastin arteriopathy that is observed in patients with SVAS and that is characterized by reduced elastin synthesis.

A similar functional deficiency in EBP has been noted (Hinek et al. 2000*a*) in Costello syndrome (MIM 218040), which is characterized by redundant skin, hyperkeratosis, facial dysmorphism, and short stature. Although the primary genetic lesion in this complex disorder is currently unknown, impaired deposition of elastin previously had been observed in multiple tissues from patients with Costello syndrome. In studying elastogenesis in skin fibroblasts from patients with Costello syndrome, therefore, Hinek and coworkers observed a disruption of the normal association of EBP and tropoelastin, as a consequence of the accumulation of chondroitin sulfate–bearing proteoglycans, an accumulation that resulted in both the shedding of EBP from the surface of Costello fibroblasts and impaired elastogenesis.

Now, in an intriguing sequel to these earlier demonstrations of impaired EBP function in diseases characterized by increased galactosugar accumulation, Hinek et al. (2000*b* [in this issue]) have demonstrated, in an article in this issue of the *Journal,* that genetic defects in the β-Gal gene that influence the expression of the alternatively spliced isoform of β-Gal, S-Gal (EBP), will result in defective assembly of elastic fibers (Hinek et al. 2000*b* [in this issue]). Multiple mutations in the β-Gal gene have previously been shown to be responsible for two clinically distinct disorders, GM1-gangliosidosis (MIM 230500, MIM 230600, and MIM 230650) and

Morquio disease type B (mucopolysaccaridosis type IVB, or MPS IVB [MIM 253010]). Using fibroblast cultures from four patients with Morquio B disease with β-Gal gene mutations encoding sequence variants in the domain common to both β-Gal and EBP (or S-Gal), as well as fibroblasts from two patients with GM1-gangliosidosis with a missense mutation, in the β-Gal gene, that does not affect the S-Gal splice variant, Hinek and colleagues looked for deficiencies in EBP and for impairment of elastic-fiber production. Very elegantly, this study confirmed that β-Gal mutants that influenced S-Gal synthesis resulted in both reduced EBP and deficient elastin synthesis and accumulation. Conversely, β-Gal mutants in skin fibroblasts from patients with GM1-gangliosidosis that were deficient in only the lysosomal variant of β-Gal and that synthesized normal levels of S-Gal demonstrated normal accumulation of insoluble elastin. Deficiencies in both S-Gal and elastin synthesis provide, therefore, a possible mechanistic explanation for the increased severity of connective-tissue disorders in patients with Morquio B disease.

Conclusions

Taken together, these three recent studies by Hinek and his colleagues emphasize that deficiencies in EBP-mediated transport of tropoelastin will impair elastogenesis and will contribute to an elastic-tissue phenotype. Hurler disease, Costello syndrome, Morquio B disease, and GM1-gangliosidosis therefore join a growing list of human elastic-fiber diseases in which the pathomechanism that leads to aberrant elastic-fiber accumulation lies in transport mechanisms secondary to fiber assembly. There will certainly be other examples of such secondary elastinopathies, and, in fact, one such emerging example is an inherited disorder of elastic fibers that is called "pseudoxanthoma elasticum," or "PXE,"in which several very recent reports have established that mutations in a gene encoding an ABC transporter, ABCC6, are responsible for defective elastogenesis (Bergen et al. 2000; Le Saux et al. 2000; Ringpfeil et al. 2000). How aberrant transport by this ABC transporter results in abnormal elastin or elastic-fiber accumulation is completely unknown at the moment, but it is to be hoped that insight into this functional relationship will provide a clearer understanding of elastic-fiber assembly, in much the same manner as Hinek et al.'s work has done to date.

Electronic-Database Information

Accession numbers and the URL for data in this article are as follows:

Online Mendelian Inheritance in Man (OMIM), http://www.ncbi.nlm.nih.gov/Omim (for congenital contractural arachnodactyly [MIM 121050], adCL [MIM 123700], MFS [MIM 154700]), SVAS [MIM 185500], Williams-Beuren syndrome [MIM 194050], Costello syndrome [MIM 218040], GM1-gangliosidosis [MIM 230500, MIM 230600, and MIM 230650], MPSI [MIM 252800], and MPS IVB [MIM 253010])

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