Elastic and Collagenous Networks in Vascular Diseases

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Abstract

Supravalvular aortic stenosis (SVAS), Marfan syndrome (MFS) and Ehlers-Danlos syndrome type IV (EDS IV) are three clinical entities characterized by vascular abnormalities that result from mutations of structural components of the extracellular matrix (ECM). Analyses of naturally occurring human mutations and of artificially generated deficiencies in the mouse have provided insights into the pathogenesis of these heritable disorders of the connective tissue. SVAS is associated with haploinsufficiency of elastin, one of the two major components of the elastic fibers. SVAS is characterized by narrowing of the arterial lumen due to the failure of regulation of cellular proliferation and matrix deposition. Mutations in fibrillin 1 are the cause of dissecting aneurysm leading to rupture of the ascending aorta. Fibrillin-1 is the building block of the microfibrils that span the entire thickness of the aortic wall and are a major component of the elastic fibers that reside in the medial layer. The vascular hallmark of EDS IV is rupture of large vessels. The phenotype is caused by mutations in type III collagen. The mutations ultimately affect the overall architecture of the collagenous network and the biomechanical properties of the adventitial layer of the vessel wall. Altogether, these genotype-phenotype correlations document the diversified contributions of distinct extracellular macroaggregates to the assembly and function of the vascular matrix.

Keywords

collagen III; elastin; fibrillin; marfan; SVAS; ESD IV

The extracellular matrix (ECM) plays a critical role in the development, growth and biomechanical properties of virtually every organ system. It follows that mutations of ECM components have detrimental effects on the assembly of individual matrices and the fitness of the whole organism. The present review focuses on ECM mutations that affect the function of the vascular system. Two ECM macroaggregates are considered, elastic and collagenous fibrils, and three conditions are discussed, supravalvular aortic stenosis (SVAS), Marfan syndrome (MFS) and Ehlers-Danlos syndrome type IV (EDS IV). Discussion of the human disorders is extended to include relevant mouse phenotypes generated by homologous gene targeting.

I. Disorders of the elastic fiber network

Elastic fibers are mainly constituted by an insoluble amorphous core of elastin and a surrounding lattice of microfibrils (Rosenbloom *et al.*, 1993). Elastin is secreted as the soluble precursor tropoelastin, which is cross-linked by the lysyl oxidase enzyme polymerizing the monomer onto a pre-existing microfibrillar matrix (Rosenbloom *et al.*, 1993). Cross-linked elastin molecules are organized in a random-coil configuration.

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Microfibrils are heterogeneous in composition and they are found in elastin containing tissues as well as in tissues that lack elastin. They are ordered in a structure that consists of repeating globular domains connected by thin fibrillar domains. The major microfibrillar components are fibrillin 1 and 2 (Sakai *et al*., 1986; Zhang *et al*., 1994).

The fibrillin proteins are nearly identical in structure, however the genes have somewhat different temporal and spatial expression patterns (Zhang *et al*., 1994; Zhang *et al*., 1995). Fibrillins are mainly composed of tandem repeats of calcium-binding epidermal growth factor-like (cbEGF) domains interspersed by cysteine-rich sequences with homology to a motif found in the TGF-β binding proteins (TB motif) or hybrids between cbEGF and TB motifs (Fib motif) (Ramirez *et al*., 1999). Head-to-tail polymerization of individual fibrillin molecules gives rise to the bead-on-a-string structure of the microfibrils. Calcium binding stabilizes the linear and rigid structure of fibrillin monomers, their interactions, the lateral packing of microfibrils and the tridimensional organization of the macroaggregates (Kielty and Shuttleworth, 1995).

Elastic fibers vary in thickness, length and tridimensional architecture depending on the direction and magnitude of the forces exerted upon the tissue. This morphological diversification can be seen in different organ systems. In the aortic wall, elastic fibers form thick concentric lamellae in the tunica media with interlaminar connecting fibers scattered radially through the vessel wall. Additionally, microfibrils are present as a complex meshwork throughout the aortic wall. It can be generalized that the elastic fibers are responsible for dilation and recoil, and the microfibrils are flexible links that make the aortic wall a working unit.

A. SVAS

SVAS is a congenital narrowing of large elastic arteries with an estimated incidence of 1 in 13,000 live births with 90% penetrance and extreme clinical variability (Morris, 1998; OConnor *et al*., 1985). The most common site of vascular stenosis is the proximal ascending aorta, however, major branches of the pulmonary artery may also be involved (Ensing *et al*., 1989). The condition can lead to systemic hypertension, cerebrovascular accident, myocardial infarction, and obstructive cardiomyopathy with heart failure. Vascular lesions shows disorganized, irregular and thickened elastic fibers; excessive, clumped and hypertrophied smooth muscle cells; extensive deposition of collagen in the inner media, and intimal fibrosis.

Elastin haploinsufficiency is sufficient for the development of SVAS (Curran *et al*., 1993; Morris, 1998). Accordingly, two alternative models have been proposed for SVAS pathogenesis. The first surmises that a quantitative deficiency of elastin may make the elastic fibers more susceptible to hemodynamic stress and damage. The second model hypothesizes that elastic fiber assembly may be influenced by the ratio of elastin to other elastic fiber components leading to the formation of deficient elastic fibers. Homologous gene targeting in the mouse has shed new light on the pathogenic processes that underlies the SVAS phenotype.

Loss of elastin gene (*Eln*) function results in mice that die perinatally of obstructive arterial disease (Li *et al*., 1998a). The arterial occlusion is caused by subendothelial cell proliferation and reorganization and proliferation of smooth muscle cells in the media (Li *et al.*, 1998a). Arterial occlusion is apparently independent of hemodynamic stress as arteries isolated in organ cultures underwent occlusion. Thus, the evidence indicates that elastin plays a regulatory role during arterial development. This is further supported by the finding that elastin haploinsufficiency in SVAS patients and in otherwise normal $Eln^{+/-}$ mice have a 2–3 fold increase in number of elastic lamellae and smooth muscle cells (Li *et al*., 1998b).

The amount of elastin therefore seems to regulate the number of lamellar units in the vessel wall which are produced during embryogenesis. A plausible new model for SVAS pathogenesis is that disruption of the biomechanical property of the vessel wall due to decreased elastin content may induce a compensatory increase in the number of lamellae ultimately leading to the occlusion of the vessel lumen.

B. MFS

MFS is an autosomal dominant disorder of connective tissue that chiefly affects the ocular, skeletal and cardiovascular system (Pyeritz and McKusick, 1979). The syndrome has high penetrance and interfamilial and, to a lesser extent, intrafamilial clinical variability. The prevalence of the disorder is 1–2 per 10,000 population with an incidence of 25% new mutations. The main vascular manifestation in MFS is dilation of the aortic root at the level of the sinuses of Valsalva. The dilated segments can progress to dissection and spontaneous rupture of the vessel wall, which is the leading cause of morbidity and mortality in MFS patients. Vascular tissues of MFS patients display disorganized and fragmented elastic fibers with excessive accumulation of amorphous matrix.

Fibrillin-1 mutations give rise to MFS. They span the entire spectrum of defects without clear relationship with the variable phenotype (Dietz and Pyeritz, 1995). The sole exception is the clustering of mutations in the middle of the molecule, exons 24–32, associated with the neonatal lethal form of MFS (Kainulainen *et al.*, 1994). Fibrillin-1 mutations interfere with calcium binding, affect the formation of intramolecular disulfide bridges within cbEGF repeats or generate truncated or internally deleted proteins (Dietz and Pyeritz, 1995). Haploinsufficiency is also a pathogenic mechanism in MFS as evidenced by the mutation in a mild form of the disease and by the results of gene targeting experiments in the mouse.

Two lines of MFS-like mutant mice have been created; they are known as the mgΔ and mgR mutant lines. The mgΔ mutation produces internally deleted fibrillin-1 molecules at a level that is ten fold less than that expressed from a normal allele. Therefore, the mgΔ mice carry a structural and hypomorphic mutation. The heterozygous mgΔ mice are morphologically and histologically normal. Homozygous mgΔ mice are indistinguishable from wild-type littermates at birth but die of vascular complications within two weeks after birth. At the site of lesions there is fragmentation of elastic fibers with reduced elastin content in the media. However, elastic fibers are histologically normal between focal lesions and in unaffected tissues. Lack of normal fibrillin-1 is therefore compatible with embryogenesis and the maturation of histologically normal elastic matrices. This implicates fibrillin-1 in maintaining homeostasis of existing elastic fibers rather than the regulation of elastic matrix assembly.

The mgR mutation produces four fold less normal fibrillin-1 molecules (Pereira *et al.*, 1999). At birth, the homozygous mgR animals are phenotypically indistinguishable from littermates. However, they gradually develop abnormalities similar to those of MFS patients and ultimately die of MFS-like vascular complications between 12 to 24 weeks of age. At 6 week of age, focal calcification of intact elastic lamellae begin to appear and to gradually increase in numbers with age. By 8 weeks, there is overt intimal hyperplasia at the calcification sites with increased and disorganized deposition of collagen and elastin. As early as 8 weeks, monocytes begin to infiltrate the medial layer followed by adventitial inflammation with fibroblast hyperplasia. At about this time fragmentation of the elastic lamellae becomes evident with concurrent loss of elastin content and aneurysmal dilatation. These data suggest that inflammation-mediated matrix proteolysis accelerates the progression of aortic aneurysm, a finding supported by the documented matrix proteolysis in aortic aneurysms and diseased valves of MFS patients (Segura *et al.*, 1998).

The gene targeting experiments together with human studies suggest that both antimorphic and hypomorphic mutations of fibrillin-1 can cause MFS since they have the same effect on the quantity of microfibrils. Accordingly, the emerging new model of MFS pathogenesis postulates that a decrease in the quantity and quality of the microfibrils below a critical threshold could lead to dissecting aneurysm. The threshold can be present perinatally due to a reduction in protein levels, or postnatally due to secondary cellular events triggered by fibrillin-1 hypomorphism.

II. Disorders of the collagenous network

Types I and III collagens are the major ECM components of the mature aorta but only mutations in collagen III are associated with vascular diseases. Type III collagen is a homotrimer of α subunits that are synthesized as procollagen precursors (van der Rest and Garrone, 1991). The three pro-α chains self-assemble intracellularly at the C-terminus and nucleate into a triple-helix in an N-terminal direction. The α chains undergo complex post-translational modifications that terminate concomitantly with the formation of the triple-helix. The formation of a properly folded and stable procollagen depends upon several structural elements, including the presence of glycine at every third amino acid residue and the hydroxylation of proline and lysine residues at the Y position of the repeating–Gly-X-Y-sequences (van der Rest and Garrone, 1991). After secretion, procollagens are processed to mature insoluble collagens that aggregate laterally to form banded fibrils.

Type III collagen fibrils–also known as reticular fibrils–are relatively more abundant in tissues subjected to periodic stress, such as the vasculature. Type III molecules participate in the tridimensional organization of type I collagen networks. Collagen fibers surrounding fibroblast of the adventitial layer are the primary source of the tensile strength of the vessel wall.

A. EDS IV

EDS IV is a rare autosomal dominant disorder characterized by thin and translucent skin with visible veins, and arterial, bowel and uterine rupture. The vascular wall rupture can occur along the entire length of the aorta including muscular arteries. However, the incidence of saccular aneurysms is very low since the vessels may not significantly dilate prior to rupture (Barabas, 1972). Histologically, there is fragmentation and thinning of the internal elastic lamina (Pope *et al.*, 1977). Life expectancy is dramatically decreased, with approximately 50% of patients having a fatal complication prior to the age of 40.

Type III collagen mutations in EDS IV include the whole spectrum of structural abnormalities, such as deletion, missplicing and missense mutations (Byers, 1995). Fibroblasts from EDS IV patients display enlarged and congested rough endoplasmic reticulum (RER) due to failure of intracellular trafficking of the mutant protein impairing its secretion (Byers, 1994). The mutant monomers can disrupt the formation or the stability of triple helices leading to miss folded or temperature-labile triple helices causing the accumulation of defective trimers in the RER (Byers, 1994). As a consequence there is deficiency of type III collagen in the extracellular matrix. The accumulation of congested abnormal material in the RER may also impede the processing and trafficking of normal molecules. It is possible that some mutant homotrimers get secreted but the association of abnormal and normal triple-helices could form fibrils that are structurally and functionally deficient.

The evidence suggests that mutations in the triple-helical domain of the α1(III) chain could predispose to structural and functional defects of homotrimers (Byers, 1994). The absence of null alleles in EDS IV patients may indicate that type III collagen haploinsufficiency is not

sufficient to cause the disorder. Consistent with the human findings, α1(III) collagen haploinsufficiency in the mouse has no apparent effect on the fitness and survival of $Col3a1^{+/-}$ animals. By contrast, $Col3a1^{-/-}$ animals die perinatally with clinical manifestations of EDS IV patients. They include rupture of the major blood vessels, spontaneous skin lesions, and frequent intestinal enlargement with occasional rupture. Ultrastructural analyses of the vasculature has revealed that type I collagen fibers in the adventitia of $Col3a1^{-/-}$ are reduced in number and are twice as large as those in the wild-type tissues. Disorganized and larger type I collagen fibers were also observed in the heart, skin intestine and lung of homozygous mutant mice. These findings gave indirect support for the dominant-negative mechanism of pathogenesis of α1(III) collagen mutations in EDS IV. They also demonstrated that the type III collagen molecules are essential components of the vascular ECM that regulate type I collagen fibrillogenesis.

III. Conclusion

The genotype-phenotype correlations discussed in this review clearly demonstrate that extracellular macro-aggregates contribute differently to the assembly and function of the vessel wall. The contributions include stabilizing molecular and cellular interactions guiding cell matrix cross-talks during morphogenesis, and providing tensile strength and elasticity to the whole ECM/cell system of the vessel wall. This diversified range of contributions is played out within the dynamic context of regional and temporal functional constrains. Appreciating and further understanding the structural and regulatory roles of the ECM therefore remains a critically important aspect of the study of mammalian development and the genesis of human disorders.

Abbreviations

ECM	extracellular matrix
SVAS	Supravalvular aortic stenosis
MFS	Marfan synd rome
EDS IV	Ehlers-Danlos syndrome type IV
cbEGF	calcium binding epidermal growth factor-like domain
TB	TGF-β binding protein
Fib	hybird cbEGF/TB motif
ELN	elastin
Col	Collagen

References

Barabas AP. Vascular complications in the Ehlers-Danlo*s* syndrome, with special reference to the arterial type or Sacks syndrome. J Cardiovasc Surg 1972;13:160–167. [PubMed: 5034837]

Byers PH. Ehlers-Danlos type IV: a genetic disorder in many guises. J Invest Dermatol 1995;105:311–313. [PubMed: 7665905]

Byers PH. Ehlers-Danlos syndrome: recent advances and current understanding of the clinical and genetic heterogeneity. J Invest Dermatol 1994;103:47S–52S. [PubMed: 7963684]

Curran ME, Atkinson DL, Ewart AK, Morris CA, Leppert MF, Keating MT. The elastin gene is disrupted by a translocation associated with supravalvular aortic stenosis. Cell 1993;73:159–168. [PubMed: 8096434]

Dietz H, Pyeritz R. Mutations in the human gene for fibrillin 1 (FBN1) in the Marfan syndrome and related disorders. Hum Mol Genet 1995;4:1799–1809. [PubMed: 8541880]

Ensing GJ, Schmidt MA, Hagler DJ, Michels VV, Carter GA, Feldt RH. Spectrum of findings in a family with nonsyndromic autosomal dominant supravalvular aortic stenosis: a Doppler echocardiographic study. J Am Coll Cardiol 1989;13:413–419. [PubMed: 2913119]

Kainulainen K, Karttunen L, Puhadda L, Sakai L, Peltonen L. Mutations in the fibrillin gene responsible for dominant ectopia lentis and neonatal Marfan syndrome. Nature Genet 1994;6:64–69. [PubMed: 8136837]

Kielty CM, Shuttleworth CA. Fibrillin-containing microfibrils: structure and function in health and disease. Int J Biochem Cell Biol 1995;27:747–760. [PubMed: 7584608]

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 1998a;393:276–280. [PubMed: 9607766]

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 1998b; 102:1783–1787. [PubMed: 9819363]

Morris CA. Genetic aspects of supravalvular aortic stenosis. Curr Op Cardiol 1998;13:214–219.

O'Connor WN, Davis JB Jr, Geissler R, Cottrill CM, Noonan JA, Todd EP. Supravalvular aortic stenosis: clinical and pathologic observations in six patients. Arch Path Lab Med 1985;109:179–185. [PubMed: 3838444]

Pereira L, Lee SY, Gayraud B, Andrikopoulos K, Shapiro SD, Bunton T, Biery NJ, Dietz HC, Sakai LY, Ramirez F. Pathogenic sequence for aneurysm revealed in mice underexpressing fibrillin 1. Proc Natl Acad Sci USA 1999;96:3819–3823. [PubMed: 10097121]

Pope FM, Martin GR, McKusick VA. Inheritance of Ehlers-Danlos type IV syndrome. J Med Genet 1977;14:200–204. [PubMed: 881711]

Pyeritz RE, McKusick VA. The Marfan syndrome: diagnosis and management. New Engl J Med 1979;300:772–777. [PubMed: 370588]

Ramirez F, Pereira L. Molecules in focus: the fibrillins. Int J Biochem Cell Biol 1999;31:255–259. [PubMed: 10216958]

Rosenbloom J, Abrams WR, Mecham R. Extracellular matrix: The elastic fiber. FASEB J 1993;7:1208–1218. [PubMed: 8405806]

Sakai L, Keene DR, Engvall E. Fibrillin, a new 350 kD glycoprotein, is a component of extracellular microfibrils. J Cell Biol 1986;103:2499–2509. [PubMed: 3536967]

Segura AM, Luna RE, Horiba K, Stetler-Stevenson WG, McAllister HA, Willerson JT, Ferrans VJ. Immunohistochemistry of matrix metalloproteinase and their inhibitors in thoracic aortic aneurysms and aortic valves of patients with Marfan's syndrome. Circulation 1998;98II:331–338.

Van der Rest M, Garrone R. Collagen family of proteins. FASEB J 1991;5:2814–2823. [PubMed: 1916105]

Zhang H, Apfelroth SD, Hu W, Davis EC, Sanguineti C, Bonadio J, Mecham RP, Ramirez F. Structure and expression of fibrillin-2 a novel microfibrillar component preferentially located in elastic matrices. J Cell Biol 1994;124:855–863. [PubMed: 8120105]

Zhang H, Hu F, Ramirez F. Developmental expression of fibrillin genes suggests heterogeneity of extracellular microfibrils. J Cell Biol 1995;129:1165–1176. [PubMed: 7744963]