Fibrillin microfibrils: multipurpose extracellular networks in organismal physiology

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Elastogenesis in Normal and Diseased Conditions

Elastic fibers are made of an insoluble amorphous core of cross-linked elastin and a surrounding lattice of microfibrils (10). Microfibrils are heterogeneous in composition and can also form macroaggregates devoid of elastin. Integral and associated components of the microfibrils include the superfamilies of fibrillins and latent TGFβ-binding proteins (LTBPs), as well as the structurally unique families of fibulins, emilins, microfibrillar-associated glycoproteins (MAGPs), and microfibrillar-associated proteins (MFAPs). Elastogenesis begins at midgestation and proceeds until completion of postnatal growth, and it involves the organized deposition by mesenchymal cells of several macromolecules that self-assemble into microfibrils and elastic fibers (10).

Fibrillins-1 and -2 (FBN1 and FBN2) are the major building blocks of extracellular microfibrils and the defective gene products in Marfan syndrome (MFS) and congenital contractual arachnodactyly (CCA), respectively (4, 10). Fibrillins are synthesized prior to tropoelastin deposition and polymerize into a characteristic “beads-on-a-string” structure, which gives rise to the microfibril lattice by lateral association of the polymers and probably, by inclusion of other structural components (Fig. 1). Microfibrils and elastic fibers are organized into tissue-specific architectures that reflect the mechanical demands of individual organ systems (10, 4). In the skin, microfibrils extend from the basement membrane of the dermal/epidermal junction into the reticular dermis, where they run parallel to the epidermis together with elastic fibers. This loosely organized network of microfibrils and elastic fibers confers pliability to the skin. In the eye, parallel bundles of microfibrils anchor the lens and adjust its thickness by conducting tension from the ciliary body. In the aorta, microfibrils associate with elastin in the tunica media to form the concentric lamellae that separate individual vascular smooth muscle cell (VSMC) layers and which confer elasticity to the aortic wall. Additionally, microfibrils devoid of elastin stabilize the tissue by connecting lamellar rings to one another, to VSMC, and to the subendothelial basement membrane.
Recent genetic studies have revealed that elastogenesis is a more complex process than previously thought. The finding that fibulin-5 (FBLN5)-null mice exhibit phenotypic abnormalities resulting from disrupted elastogenesis has implicated this molecule in the early assembly of the elastic fiber (16, 21). Specifically, FBLN5 is believed to regulate assembly by providing the molecular bridge between tropoelastin in the pericellular space and integrins on the cell surface. The phenotype of the FBLN5-null mouse includes loose skin, pulmonary emphysema, vascular malformations, and progressive narrowing of the proximal aorta. These manifestations closely replicate the severe recessive form of cutis laxa, a human connective tissue disorder that is similarly caused by FBLN5 mutations (14).

The enzyme lysyl oxidase (LOX) mediates the extracellular conversion of tropoelastin molecules into the insoluble elastic meshwork (10). Concordant with this fact, genetic disruption of LOX activity in mice leads to reduced elastin cross-linking and fragmented elastic fiber architecture; as a result, the mutant mice manifest severe cardiovascular instability, in the form of ruptured arterial aneurysm, and perinatal death (6, 15). There are four LOX-like proteins (LOXL1–4) in the mammalian genome, and at least one of them (LOXL1) has been implicated in elastogenesis (13). Mice lacking the LOXL1 gene in fact develop abnormalities in several elastic tissues as a result of abnormal accumulation of tropoelastin. The manifestations include enlarged air space of the lung, loose skin, vascular defects, and pelvic organ prolapse. Biochemical and immunolocalization data indicate that interaction between LOXL1 and FBLN5 juxtaposes the enzyme and the tropoelastin substrate for efficient and spatially restricted polymer formation (13). That elastic fiber defects generally appear later in LOXL1- than FBLN5-null mice argues for a predominant role of the former gene product in adult elastic tissue homeostasis.

Mice without elastin (ELN) recapitulate the phenotype of ELN haploinsufficiency in human supravalvular aortic stenosis (SVAS). These features include abnormalities in the aortic wall and altered hemodynamics associated with changes in wall compliance (5, 11). ELN-null mice survive gestation but die postnatally from subendothelial accumulation of VSMC that ultimately occlude the vascular lumen. As in human SVAS, the vessel wall of ELN haploinsufficient mice displays an increased number of elastic lamellae, suggesting that the deficiency affects normal vascular development (12). The vascular phenotypes of the ELN mutant mice are accounted for by defective cell-matrix interactions that normally regulate VSMC migration, induce a quiescent phenotype, and inhibit proliferation (9).

Microfibrils and Elastogenesis

Genetically targeted strains of mice have been created that replicate the neonatal lethal or clinically progressive forms of MFS. Mice homozygous for a severely hypomorphic Fbn1 allele (mgR) die between 9 mo and 1 yr of age, due to aortic dissection and rupture, and replicate the clinically progressive form of MFS (18). That the elastic fibers of these mutant mice show normal morphology at birth suggested that aneurysm
progression is largely driven by secondary cellular events (3, 18). Specifically, the first detectable alteration in mgR homozygotes is loss of the connecting filaments that normally serve as a structural interface between elastic lamellae and neighboring VSMC. Next, VSMC begin secreting multiple matrix components as well as matrix-degrading enzymes in an abortive attempt to remodel a defective tissue. The net result of the defective remodeling is the initiation of local elastolysis, which is temporally correlated with elastic fiber calcification and immediately followed by inflammatory infiltration. The latter process begins at the adventitial surface and progresses into the media and is temporally and spatially correlated with structural collapse of the vessel wall, aneurysm formation, and dissection. These observations imply that fibrillin-1 microfibrils serve an essential role in elastic fiber homeostasis during extra-uterine life. As such, they offer the potential for therapeutic intervention aimed at blunting the elastolysis that is initiated by VSMC and exacerbated by vessel wall inflammation.

Mice harboring a heterozygous cysteine substitution (C1039G allele) in a calcium-binding EGF motif of fibrillin-1 or harboring only one functional FBN1 gene show some of the above abnormalities but do not progress to the point of dissection and death (19; F. Ramirez, unpublished observations). Homozygous C1039G mutant mice and FBN1-null mice, on the other hand, complete embryonic development and die soon after birth due to failure of the vessel wall without antecedent calcification or inflammation (19; F. Ramirez, unpublished observations). Together, these data equate the consequences of qualitative and quantitative mutations in MFS pathogenesis; indicate that the relative abundance of functionally competent fibrillin-rich microfibrils determines the clinical severity of MFS; and demonstrate that elastogenesis proceeds despite severe quantitative and/or qualitative deficits in fibrillin-1.

Microfibrils and Growth Factor Signaling

FBN1-deficient mice display diffuse distal air space enlargement by 7–9 days of age in the absence of tissue destruction or inflammation (17, 1). This phenotype, which resembles the emphysema-like phenotype of some MFS patients, is associated with dysregulated TGFβ signaling. TGFβs are multipotent cytokines that are synthesized as inactive precursor molecules containing an NH$_2$-terminal prodomain termed latency associated peptide (LAP) (20). LAP remains associated with TGFβ (constituting the small latent complex) and ultimately becomes covalently linked to a latent TGFβ binding protein (LTBP)-1, -3, or -4 to form the large latent complex. It is currently believed that LTBP binding facilitates growth factor secretion. The LTBP s are structurally related to the fibrillins and participate in TGFβ regulation, in part by targeting TGFβ complexes to the ECM (7). Hence, morphogenetic abnormalities in the microfibril-deficient state may result in incorrect latent complex sequestration and consequently, lead to excessive activation of and signaling by the growth factor. The demonstration that LTBP bind directly to fibrillin-1 and that antagonism with TGFβ-neutralizing antibody rescues lung morphogenesis is consistent with this model (1, 2).

Additional support for the involvement of microfibrils in regulating growth factor signaling derives from the analysis of FBN2-null mice (2). Like CCA patients, these mutant mice display transient contractures of small and large joints; they also exhibit a limb patterning defect in the form of bilateral syndactyly. The defect correlates with altered matrix assembly in the developing autopod and perturbed BMP signaling. The latter correlation is based on the finding that the combination of haploinsufficient FBN2 and BMP7 mutations, which are by themselves phenotypically silent, result in impaired digit formation in the absence of additional manifestation. Hence, the
tissue-specific architecture of extracellular microfibrils appears to play a critical role in determining growth factor bioavailability.

Biological and Clinical Considerations

The above results have provided a conceptual framework to investigate ill-defined biological and pathological interactions between the architectural matrix and growth factor signaling (20). Although still in its infancy, this new model of elastic matrix physiology has already provided testable hypotheses to explain how the specificity of growth factor signaling may be compartmentalized within the extracellular space, and how its dysregulation may underlie the clinical variability usually observed in inheritable disorders of the connective tissue.

Growth factors are one of the major classes of mediators controlling cellular responses (20). They are normally secreted by cells within the responding tissue and act over relatively restricted distances; sometimes only a few cell diameters. Because these effector molecules have such potent actions and their receptors are widely distributed, there is a need to control growth factor bioavailability in order to ensure proper spatio-temporal signaling, as well as to achieve a sufficient local concentration of the effector molecules (20). Tissue-specific organization of architectural macroaggregates, like microfibrils, properly position latent signals within an appropriate context that cells can effectively translate into productive responses. As such, these extracellular networks serve as interactive information highways with directional sign posts and embedded signals, in addition to being structural scaffolds imparting tissue integrity (Fig. 2). It follows that mutations perturbing the normal architecture of the ECM can have a wide range of deleterious consequences on tissue integrity and function, as well as organ formation and growth and tissue homeostasis. This last postulate in turn implies that additional and previously unsuspected biological targets may exist, which can be exploited with novel therapies that counteract the pathological progression of inheritable and acquired diseases of the connective tissue.

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REFERENCES