Elastic fibers and biomechanics of the aorta: Insights from mouse studies

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Abstract

Elastic fibers are major components of the extracellular matrix (ECM) in the aorta and support a life-long cycling of stretch and recoil. Elastic fibers are formed from mid-gestation throughout early postnatal development and the synthesis is regulated at multiple steps, including coacervation, deposition, cross-linking, and assembly of insoluble elastin onto microfibril scaffolds. To date, more than 30 molecules have been shown to associate with elastic fibers and some of them play a critical role in the formation and maintenance of elastic fibers in vivo. Because the aorta is subjected to high pressure from the left ventricle, elasticity of the aorta provides the Windkessel effect and maintains stable blood flow to distal organs throughout the cardiac cycle. Disruption of elastic fibers due to congenital defects, inflammation, or aging dramatically reduces aortic elasticity and affects overall vessel mechanics. Another important component in the aorta is the vascular smooth muscle cells (SMCs). Elastic fibers and SMCs alternate to create a highly organized medial layer within the aortic wall. The physical connections between elastic fibers and SMCs form the elastin-contractile units and maintain cytoskeletal organization and proper responses of SMCs to mechanical strain. In this review, we revisit the components of elastic fibers and their roles in elastogenesis and how a loss of each component affects biomechanics of the aorta. Finally, we discuss the significance of elastin-contractile units in the maintenance of SMC function based on knowledge obtained from mouse models of human disease.

Introduction

Elastic fibers are required for expansion and recoil of the aortic wall and alteration in quantity or quality of elastic fibers exhibits profound effects on the integrity and biomechanics of the aorta. The aorta is the first segment of the arterial tree and receives blood from the heart. In order to maintain a stable supply of blood to peripheral organs throughout the cardiac cycle, elasticity of the proximal ascending aorta plays a critical role in storing a portion of blood during systole and sending blood out during diastole, exerting the Windkessel effect [1]. The aorta and large elastic arteries are unique in that they contain well-defined elastic lamellar units, in which elastic lamella composed of elastic fibers alternate with vascular smooth muscle cells (SMCs) throughout the medial region. Within each lamellar unit, elastic fibers and SMCs maintain close contacts through connecting filaments that are formed by elastin extensions, thereby keeping SMCs and elastic lamella in a circumferential orientation [2]. These connections allow transmission of mechanical forces generated by the cyclic pumping of blood to the SMCs (outside-in signals), as well as transmission of tension generated by actomyosin within the SMCs to be delivered to the elastic lamella (inside-out signals), thereby forming “elastin-contractile units”. Hence, elastic fibers serve as a mediator of dynamic mechanical signals in the blood vessel wall.
The molecular mechanism of elastic fiber formation has begun to be elucidated through the identification of molecules associated with elastic fibers and their loss-of-function analyses in mice with distinct elastic fiber defects [3–5]. Elastic fiber formation occurs via multiple steps in a tightly regulated manner which actively takes place from mid-embryogenesis throughout early postnatal life [6,7]. Elastic fibers formed during this period last one’s lifespan. Once elastic fibers are degraded by aging or lost by injuries, they do not regenerate spontaneously, thus compromising the biomechanical properties of the aortic wall. Here, in attempt to provide knowledge on the detailed molecular mechanism of elastic fiber assembly and its role on vessel mechanics in vivo, we first review the components of elastic fibers and provide an updated view of elastic fiber formation, and then we discuss the impact of the loss of major elastic fiber components on the biomechanics of the thoracic aorta. Lastly, we discuss the importance of elastin-contractile units and their association with aortic diseases.

Molecular players in elastic fiber assembly

Elastic fibers are comprised of an elastin core and surrounding microfibril scaffold. Elastin is encoded by a single ELN gene and is translated to a ~70 kDa protein secreted as the monomeric form called tropoelastin from elastogenic cells in the skin, lung and vasculature [8]. Elastin contains alternating hydrophobic and cross-linking domains that are responsible for self-aggregation and formation of cross-links, respectively [9]. Coacervation of tropoelastin, which induces self-aggregation and phase separation, occurs spontaneously under physiologic conditions and is important for alignment and cross-linking of tropoelastin [10,11]. However, coacervation alone is not sufficient for polymerization of elastic fibers, which requires the action of cross-linking enzymes. Lysyl oxidase (LOX) and lysyl oxidase-like 1 (LOXL1) belong to a multigene family consisting of five members (LOX and LOXL1 to 4) and are copper- and lysyl-tyrosyl-quinone (LTQ)-dependent secreted enzymes [12,13]. LOX and LOXL1 are the most well-studied enzymes of the family that contain an N-terminal propeptide, which is cleaved during proteolytic activation, and a conserved catalytic domain. LOX and LOXL1 mediate cross-linking of elastin by catalyzing the oxidative deamination of peptideyl lysine residues, subsequently forming the covalent elastin-specific cross-links desmosine and isodesmosine. LOX also catalyzes cross-linking of collagen. Recent crystal structure analysis of human LOXL2 revealed a conserved catalytic domain among the LOX family and showed generation of LTQ upon copper-mediated activation of the enzyme [14]. It has been shown in vitro that LOX-mediated cross-linking of tropoelastin occurs simultaneously with elastin deposition onto microfibrils [15].

Filbin-4 (encoded by Efemp2) and filbin-5 (Fbln5) are secreted glycoproteins with a modular structure. They are expressed in the aorta and are essential for elastic fiber formation in vivo [3–5]. The fibulin family is comprised of seven members and fibulin-4 through fibulin-7 belong to the short fibulins (reviewed in [16,17]). Among short fibulins, fibulin-4 and fibulin-5 are known as elastogenic fibulins. Filbin-5 was first reported as an elastin binding protein and its deletion in vivo caused systemic elastic fiber defects in mice [3,4]. Subsequently, it was shown that filbin-5 potentiates coacervation of tropoelastin as well as limits the size of coacervated elastin [18,19]. Consistently, electron microscopy analysis showed that Fbln5-null (Fbln5−/−) skin contains large aggregates formed by insoluble elastin [20]. Filbin-4 shares high homology with fibulin-5 and also binds tropoelastin in vitro [21]. Deletion of fibulin-4 in mouse (Fbln4−/−) causes a more severe phenotype than the Fbln5−/− mouse, exhibiting aortic rupture, diaphragm hibernation and perinatal lethality, which resembles Lox-null (Lox−/−) pups [5,22–24]. Both fibulins have distinct roles in elastogenesis. Biochemical analyses showed that the N-terminal region of fibulin-4 binds a propeptide of LOX and facilitates tropoelastin binding to LOX [18], whereas fibulin-5 binds LOXL1 via the C-terminal domain and facilitates tethering of LOXL1 onto elastic fibers [25] and/or proteolytic activation of preproLOXL-1 to mature LOXL1 [20]. Taken together, fibulin-4 and fibulin-5 play a critical role in optimizing the conditions for tropoelastin cross-linking through regulation of coacervation and interactions with LOX and LOXL1, respectively.

Visible at the periphery of the elastin core are microfibrils that act as a pre-existing scaffold for elastin polymer deposition as well as regulation of growth factors and cytokines. Microfibrils are formed by polymerization of large ECM proteins, mainly fibrillin-1 (Fbn1) and fibrillin-2 (Fbn2) [26–28]. Heparan sulfate and a fibronectin network are essential for formation of the microfibril scaffold [29–31]. Fibronectin is also shown to be important for microfibril homeostasis in early passage-human skin fibroblasts [32]. A complete deletion of fibrillin-1 in mice causes neonatal lethality due to aortic aneurysm rupture and disruption of elastic fibers [33]. Further deletion of Fbn2 in the Fbn1-null (Fbn1−/−) background in mice worsens the phenotype and induces embryonic lethality with poorly organized elastic fibers, indicating that microfibril scaffold formation is indeed a prerequisite for proper deposition of polymerized elastin [33].

Fibrinollin's role as a scaffold for elastic fiber assembly is not only through homophilic interaction-based long fibril structures, but also by providing interactions with numerous binding proteins [34–36]. Recently, more
complex interactions among elastin- and microfibril-associated proteins have been shown to play an important role in navigating assembled elastin onto microfibrils. EMILIN-1 (elastin microfibril interface-located protein), which is abundantly expressed in the aorta and bone, binds and co-localizes with fibulin-4 and is required for the deposition of fibulin-4 onto the ECM in osteoblasts [37]. Latent TGFβ binding proteins (LTBPs) belong to the fibrillin/LTBP family and are comprised of four members LTBP-1 through -4, including splice variants LTBP-4S (short form) and LTBP-4L (long form) [38]. LTBP-1, -2, and -4 directly bind to fibrillin-1 whereas LTBP-3 does not bind to fibrillin-1 in vitro. However, LTBP-3 co-localizes with microfibrils, indicating that LTBP-3 interacts with other microfibril-associated proteins [34,39,40]. LTBP-1 uses a fibronectin network for its deposition onto microfibrils and LTBP-3 and -4 associate with the fibrillin-1 network [40]. LTBP-1, -3, -4 also bind to the prodomain of TGFβ and to mature TGFβ dimer and regulate bioavailability of TGFβ in vivo [41,42]. A knockout study in vivo showed that LTBP-4 plays a crucial role in elastic fiber formation [43,44] and suggested that LTBP-4 aids in deposition of elastin onto microfibrils by interacting with fibulin-5 [45]. By using a combination of binding assays and phenotypic analyses of Ltbp4s-null (Ltbp4s−/−) and Ltbp4-null (Ltbp4−/−) mice, it was recently shown that LTBP-4L and LTBP-4S favor the interaction with fibulin-4 and fibulin-5, respectively, and the absence of both forms of LTBP-4 leads to markedly reduced expression and impaired localization of fibulin-4 and fibulin-5 on the microfibrils, resulting in elastic fiber defects with dysmorphic elastin aggregates [46,47]. Together with preferential binding between fibulin-4 and LOX and fibulin-5 and LOXL1, it is conceivable that down-regulation or absence of one of the molecules in the fibulin-4-LOX-LTBP-4L or fibulin-5-LOXL1-LTBP-4S complex affects overall assembly efficiency and the process of elastic fiber formation (Fig. 1).

Elastic fibers and biomechanics of the thoracic aorta

Scope and definition of mechanical terms

Elastic fibers provide reversible elasticity to the aorta, while collagen fibers provide strength and limit distension at high pressures. Loss of critical elastic fiber components alters the mechanical behavior of the aortic wall. Genetically-modified mice have been instrumental in determining mechanical contributions of different elastic fiber components. In this section, we summarize studies that quantify mechanical behavior of the thoracic aorta in mouse models with elastic fiber defects. In many cases, we compare structural stiffness (depends on material properties and aortic geometry) and material stiffness (depends

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*Fig. 1.* Proposed model of elastogenesis. Tropoelastin is secreted by elastogenic cells, binds to fibulins (predominantly fibulin-5), and undergoes self-aggregation (coacervation). Fibulin-5 binds to LOXL1 and fibulin-4 binds to LOX and the complexes are deposited onto microfibrils (green) with the aid of LTBP-4S and LTBP-4 L, respectively. Cross-linking and polymerization of elastic fibers proceed.

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only on material properties) between groups under physiologic loading conditions. Structural stiffness is often determined from in vitro pressure-diameter tests with the aorta held at the in vivo axial stretch ratio and is the inverse of compliance or distensibility. We present pressure-diameter data for a range of mouse models and discuss the implications of elastic fiber defects on structural stiffness under different loading conditions. Calculation of material stiffness requires information about the unloaded dimensions of the aortic wall, in addition to pressure-diameter data, and hence is not always reported.

Material stiffness depends on multiaxial deformation of the aortic wall and should be calculated by fitting a nonlinear strain energy function to biaxial mechanical testing data. However, an estimate of the circumferential material stiffness called the incremental elastic modulus can be obtained by determining the linear slope of the stress-strain curve under physiologic loading conditions. The incremental elastic modulus of the aorta for a range of animal species from shark to toad to mouse to human is around 0.4 MPa and it has been suggested that this value represents a “universal elastic modulus” that provides optimal mechanical function in a pulsatile cardiovascular system [48]. We highlight mouse models that are able to maintain the universal elastic modulus through remodeling of the aortic wall and those that cannot and are often associated with aneurysmal disease.

Additional mechanical properties compared between mouse models include residual strain (strain remaining in the aortic wall in the unloaded state due to growth and remodeling of the wall components), stored strain energy (the total energy stored due to multiaxial, physiologic deformation of the aortic wall and available to do work on the blood in vivo), and energy dissipation (energy loss due to viscous components in the aortic wall), which is the difference between the stored strain energy with loading and returned strain energy with unloading.

**Elastin**

Elastin null (Eln−/−) mice die at birth with obstructive aortic occlusion [49]. The ascending aorta from newborn Eln−/− mice has increased structural stiffness compared to Eln+/+ [50]. There is a large increase in energy dissipation in Eln−/− aorta, which is consistent with the mechanical role of elastin in the large arteries [51]. The aorta from elastin heterozygous (Eln+/−) mice has increased structural stiffness compared to Eln+/+ mice [52] and compensatory remodeling of the axial and circumferential residual strains [53]. Eln−/− mice live a normal lifespan [54]. During postnatal maturation, the increase in aortic structural stiffness precedes and may cause hypertension in Eln−/− mice [55]. Results from constitutive modeling of elastin and collagen contributions to aortic mechanics are consistent with reduced elastin amounts and predict reorganization of collagen fiber alignment and nonlinearity in Eln−/− aorta to maintain the material stiffness near wild-type levels [56]. Eln−/− mice that have been rescued by expression of human elastin through a bacterial artificial chromosome (hBACmNull) have about 30% of the normal elastin levels and even more severe increases in aortic structural stiffness and blood pressure than Eln−/− mice [57]. hBACmNull mice exhibit aortic narrowing similar to humans with supravalvular aortic stenosis (SVAS) that is caused by elastin mutations leading to reduced levels of functional elastin [58]. Aortic narrowing in hBACmNull mice may be caused by deficient circumferential growth [59].

**Fibulin-5**

Fbln5−/− mice live a normal lifespan, but have fragmented elastic fibers in elastin rich tissues [3,4]. Fbln5−/− mice have a longer aorta [4] with increased structural stiffness [3]. Biaxial mechanical testing confirms that Fbln5−/− aorta has increased structural stiffness, but similar material stiffness to wild-type aorta, as well as decreased stored strain energy and increased energy dissipation [60]. The material stiffness is maintained at similar values in Fbln5−/− and wild-type aorta throughout postnatal maturation [61], suggesting a universal elastic modulus for the aorta that is maintained even with genetic mutations that alter the available amounts and structure of elastic fiber components in the wall [48]. Fbln5−/− aorta has decreased active contractility [62], which may be related to altered coupling between SMCs and elastic fibers due to the absence of fibulin-5.

**Fibulin-4**

Fbln4−/− mice die at birth with severe vascular and lung defects [5]. Newborn Fbln4−/− mice have ascending aortic aneurysms and increased structural stiffness, but similar material stiffness to wild-type aorta [63] consistent with the hypothesized ability of the aorta to maintain material stiffness despite elastic fiber protein deficiencies. Similar to newborn Eln−/− aorta, newborn Fbln4−/− aorta shows a large increase in energy dissipation with cyclic loading [51] demonstrating that correctly assembled elastic fibers, and not just the elastin protein, are necessary for reversible elasticity of the aorta. Mutations in fibulin-4 cause cutis laxa which is often accompanied by aortic aneurysms. A mouse model with a fibulin-4 mutation (Fbln4E57K/E57K) identified in human patients with cutis laxa develops ascending aortic aneurysms in approximately half of the mice homozygous for the mutation [64]. Fbln4E57K/E57K aorta has increased structural stiffness compared to wild-type, even when an aneurysm is not present [65].

Mice with SMC specific loss of fibrillin-4 (Fbln4−/− SMKO) were developed to further investigate the role of
fibulin-4 in vascular biology. \(Fbln4^{SMKO}\) mice develop ascending aortic aneurysms [24]. Aneurysms are detectable by 2 weeks of age in \(Fbln4^{SMKO}\) mice, but increases in structural stiffness are detectable by 1 week of age [66], indicating that increases in structural stiffness precede and may play a causal role in aneurysm formation. Biaxial mechanical testing showed an increase in aortic material stiffness of \(Fbln4^{SMKO}\) mice and other mouse models of aneurysmal disease [67] suggesting that aneurysms may manifest in adult animals when the aorta is not able to maintain the universal elastic modulus. In support of this hypothesis, crossing \(Fbln4^{SMKO}\) mice with thrombospondin-null (\(Tbts1^{-/-}\)) mice rescues the aneurysm phenotype along with the material stiffness [68]. A growth and remodeling model of aortic development was used to understand how changes in elastin, collagen, and SMC amounts and behavior could lead to the resulting mechanical behavior of \(Fbln4^{SMKO}\) aortas. Reductions in elastin stress contribution, increases in collagen stress contribution, and altered SMC response to developmental changes in hemodynamic forces were required for the model to reproduce \(Fbln4^{SMKO}\) aorta mechanical behavior [69]. The changes in SMC response are consistent with changes in SMC phenotype observed in \(Fbln4^{SMKO}\) mice [24,66].

**Lysyl oxidase**

\(Lox^{-/-}\) mice die at birth with ruptured arterial aneurysms and diaphragm [22,70]. Newborn \(Lox^{-/-}\) mice have ascending aortic aneurysms, increased structural stiffness, and increased material stiffness compared to wild-type aortas [71]. The increased material stiffness in \(Lox^{-/-}\) aortas contrasts with the normal material stiffness in \(Fbn1^{-/-}\) aortas [63]. Since lysyl oxidase crosslinks both elastin and collagen, it is possible that the compensatory changes in collagen arrangement and nonlinearity hypothesized to maintain the universal elastic modulus in other mouse models of elastic fiber defects (i.e. \(Eln^{-/-}\) [56] and \(Fbn1^{-/-}\) [61]) cannot occur in \(Lox^{-/-}\) aortas. Like newborn \(Eln^{-/-}\) and \(Fbn1^{-/-}\) aortas, newborn \(Lox^{-/-}\) aortas show a large increase in energy dissipation confirming that effective elastic fiber crosslinking is necessary for reversible elasticity in the aorta [51]. Mutations in lysyl oxidase have been identified in human patients with thoracic aortic aneurysms [72]. A mouse model with a mutation identified in humans (LOX p.M298R) shows perinatal lethality and large aneurysms in mice homozygous for the mutation. Mice heterozygous for the mutation live a normal lifespan, but have increased structural stiffness in the aorta [73]. Chemical inhibition of LOX with BAPN induces thoracic aortic aneurysm and dissection in mice [74]. SMCs in BAPN treated aorta are characterized by mechanical stress induced apoptosis, indicating that changes in mechanical behavior of the aorta due to reduced elastic fiber crosslinking may alter SMC mechanobiology and contribute to aneurysm formation and dissection [75].

**Fibrillin-1**

Homozygous mice with deletion of a large portion of the fibrillin-1 gene and insertion of a neomycin resistance cassette (\(Fbn1^{mgR/mgR}\)) die before three weeks of age from cardiovascular complications [76]. Mice that underexpress fibrillin-1 due to insertion of a neomycin resistance cassette, without deletion of any fibrillin-1 gene segments (\(Fbn1^{mgR/mgR}\)) die at about 4 months of age with large aortic aneurysms [77]. \(Fbn1^{mgR/mgR}\) ascending aorta has increased structural and material stiffnesses, even when an aneurysm is not present [78], consistent with the hypothesis that aneurysms may manifest when the adult mouse aorta is not able to maintain the universal elastic modulus. Similar to \(Fbn5^{-/-}\) aorta, \(Fbn1^{mgR/mgR}\) aorta has reduced stored strain energy and increased energy dissipation [78].

**Comparison of aortic biomechanics and elastin/collagen contributions in different mouse models**

Pressure-diameter tests at the in vivo axial stretch were performed in many of the mouse models discussed above and are summarized in Fig. 2. The structural stiffness (or local slope of the curve) is often affected by elastic fiber defects, but is highly dependent on the applied pressure. Due to the pressure dependence, an aorta from a hypertensive mouse (such as \(Eln^{-/-}\)) will have increased structural stiffness in vivo, even with no changes in the geometry and material properties of the aortic wall [79]. The applied pressure at which the curve transitions between low and high structural stiffness is also affected by elastic fiber defects. The structural stiffness at low pressure is associated with elastin resistance to deformation, while the structural stiffness at high pressure is associated with collagen resistance to deformation [80]. For many of the mouse models, there are not large differences in the structural stiffness at low pressure where elastin dominates, implying that many genetic modifications (including \(Eln^{-/-}\) and \(Fbn5^{-/-}\)) have little effect on the low-pressure mechanical behavior attributed to elastin. The more severe mouse models that show highly fragmented elastic fibers and develop aortic aneurysms (including \(Fbn4^{SMKO}\) and \(Fbn1^{mgR/mgR}\)) have decreased stiffness at low pressure, consistent with reduced mechanical contributions of elastin.

Interestingly, all of the mouse models with elastic fiber defects have increased stiffness in the high-pressure region attributed to collagen. They also transition to the stiffer region at lower pressures than wild-type aorta. Some models, such as \(Fbn4^{SMKO}\) [81], have collagen defects in addition to elastin
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defects that may explain the changes in mechanical behavior at high pressure. Others, such as *Elm⁻/⁻* [52,56], do not have measurable changes in collagen amounts or organization. Experiments with elastase degradation and results from constitutive modeling of aortic wall components suggest that elastic fibers hold collagen fibers in compression, resulting in wavy collagen fibers, and allowing them to deform significantly before contributing to mechanical resistance [82,83]. The loss of functional elastic fibers may decrease collagen fiber waviness, so the collagen fibers are straighter and contribute to the mechanical resistance at a lower point in the pressure-diameter curves. The loss of functional elastic fibers may also reduce the variation in collagen fiber waviness so that most collagen fibers are recruited at the same deformation, leading to a sharp rather than gradual increase in structural stiffness. These observations imply that changes to elastin and collagen (and SMCs) must be considered together to fully understand the mechanical implications of elastic fiber defects.

**Biological significance of elastin contractile units**

SMCs possess an intrinsic contractile machinery to respond to mechanical loads. The contractile machinery is indirectly connected to elastic fibers to form an elastin-contractile unit, which is the physical and functional unit from elastic fibers to actin contractile filaments that allows the coordinated response of aortic wall components to mechanical stress [2] (Fig. 3). SMCs do not form sarcomeres like cardiac or skeletal muscle cells and contraction is regulated mainly by a ligand-induced, receptor-mediated signaling pathway involving the mobilization of intracellular calcium and contractile filaments (reviewed in [84]). SMCs organize contractile filaments comprised of smooth muscle (SM) actin and SM myosin in a diagonal direction and they connect to the dense plaque (DP), where elastic fibers attach to integrins from outside of the cell and form focal adhesions (FAs) underneath the cell membrane [85]. Actin isoforms that form contractile filaments in large arteries are mainly α SM actin [86], whereas nonmuscle γ actin forms cytoskeleton near the cortex of SMCs [87]. Actin molecules in non-contractile filaments are more dynamic compared to contractile filaments, but both contractile filaments and the cortically-distributed actin cytoskeleton generate tension in SMCs [88].

Recently, it has been shown that genetic mutations in the components of elastin-contractile units are responsible for thoracic aortic aneurysms (TAAs) (reviewed in [89]). The mutations are largely divided into two groups; the first group includes genes encoding for extracellular proteins that constitute or are associated with microfibrils such as *FBN1* [90], *LOX* [72,73], *EFEMP2* [91], microfibril associated protein 5 (*MFAP5*) [92], elastin microfibril interface 1 (*EMILIN1*) [93] and *LTBP3* [94]. The bundles of microfibrils are initially linked to SMCs, then elastin progressively infiltrates the microfibrils to form elastin extensions [2]. Fbn1 interacts with integrin α5β1 and αvβ3 via its RGD domain [95] and downregulation of Fbn1 in mouse (*Fbn1<sup>−/−</sup>*/mgR) markedly reduces phosphorylation of focal adhesion kinase (FAK) in cardiac myocytes and leads to dilated cardiomyopathy [96]. In addition, compound heterozygous mice for Fbn1 and β1 integrin (*Fbn1<sup>+/−</sup>*/β1<sup>−/−</sup>)...
Itgb1+/−) recapitulate the phenotype of Fbn1<sup>mgR/mgR</sup> mice, suggesting that Fbn1 may be crucial to signal activation in SMCs mediated by elastin extensions and integrins. Interestingly, mutations in Eln [58] or Fbn5 [97] that form the elastin core do not cause aortic aneurysms; rather, they develop supravalvular aortic stenosis (SVAS) and tortuous elongated aorta, respectively, exhibiting morphogenetic defects due to impaired elastic fibers in the aorta.

Focal adhesions were described as electron-dense regions in SMCs where elastin extensions attach to the outside of the cell and actin filaments anchor obliquely at the cytoplasmic side underneath the cell membrane [98]. Although mutations in the components of focal adhesions have not been reported in aortic aneurysms, studies in mice have implicated their role in aortic homeostasis. Mechanical force in the aorta is transmitted to focal adhesions via heterodimeric α- and β-integrins and regulates actin filaments. Several key molecules have been identified within focal adhesions that are involved in mechanotransduction (reviewed by [99,100]). Integrin linked kinase (ILK) is a scaffold protein that associates with the cytoplasmic tail of β integrin and forms the IPP complex with cysteine-rich protein (PINCH) and parvin and links to actin filaments [101]. Deletion of Ilk in SMCs results in aneurysmal dilatation of the aorta [102] and deletion in neural crest cells shows reduced phosphorylation of pSmad3 and downregulation of SMC markers with aneurysm development [103]. Talin serves as a mechanosensitive molecular hub transducing mechanical force to actin filaments and converting it into biochemical signals (reviewed in [104]). Activation of talin, which binds the β integrin tail, leads to conformational change of the integrin into the active form, and tension generated by actin filaments can stabilize integrins together with talin in its extended open form [105]. Recently, downregulation of talin has been reported in SMCs harvested from patients with aortic dissection, along with increased proliferation and migration [106].

The second group for which elastin-contractile unit mutations are reported in TAAs includes genes
encoding for the components of actomyosin contractile filaments and their regulators in SMCs (reviewed in [107]). Mutations in SM-myosin heavy chain (encoded by MYH11), which causes deletion in the C-terminal coiled-coil domain, is predicted to exert a dominant negative effect on myosin motor activity [108]. In addition, 3 missense variants (L1264P, R1275L, R712Q) and 1 rare variant (R247T) in MYH11 have been identified and suggested to damage coiled-coil domain (L1264P), affect the ATPase region (R712Q) and disrupt the myosin motor activity (R247T) [109]. More than 40 mutations in ACTA2 have been identified in familial TAA and dissection [110] and R179H and R258C mutant actin have been shown to exhibit reduced FBN1 deposition, increased TGF-β, and increased SMC contractile proteins, but disrupting the myosin motor activity (R247C) [109].

Concluding remarks and future directions

Studies in knockout mice have been instrumental in defining critical players in elastic fiber assembly and identifying molecules that work together to regulate elastin coacervation, cross-linking, deposition onto microfibrils, and interactions with SMCs. Additional in vivo and in vitro studies are needed to fully understand the role(s) of the over 30 different molecules implicated in elastic fiber synthesis so that we may someday be able to encourage elastic fiber repair or de novo synthesis in the case of defects or damage caused by disease or aging. It is important to remember that elastic fiber synthesis occurs in a dynamic mechanical environment, with the aortic wall distending and recoiling with each heartbeat, so that inside out and outside in mechanical signaling likely contribute to the regulation of elastic fiber synthesis in development and dysfunction with disease.

The mechanical data on different mouse models of elastic fiber defects show that increased aortic structural stiffness is a common consequence of reduced elastin levels or misassembled elastic fibers, but that increased aortic material stiffness generally manifests in extreme cases that are associated with aneurysmal disease. The results suggest that failure of the aortic wall to maintain the universal elastic modulus has downstream effects on SM phenotype that may be caused by the microenvironment within the stiffened ECM. Future work to quantify the material behavior of the aorta under physiologic loading conditions and the resulting SM phenotype in different mouse models with elastic fiber and other ECM defects is needed to further investigate this hypothesis and understand the limits and consequences of maintaining the universal elastic modulus. When reported, stored strain energy is reduced and energy dissipation is increased in aortas with elastic fiber defects. These energetic alterations increase the work required by the heart to circulate blood and may contribute to secondary cardiovascular diseases associated with elastic fiber defects.

Elasticity is an essential biomechanical property of the aorta and is provided by elastic fibers and enhanced by elastin-contractile units. Whereas insoluble polymerized elastin confers dynamic elasticity and recoil to the aortic wall, elastin extensions aid in connecting elastic lamella to integrins and transmit mechanical forces through focal adhesions, thereby converting the physical stimuli into biochemical signaling and regulating actin contractile filaments. We hypothesize that a homeostatic stress state, determined by external forces from ECM connections and internal forces from contractile filaments, is necessary for maintenance of the SM phenotype. In the case of genetic defects to elastic fiber proteins, microfibrils may still be in place, but elastin extensions are disrupted, interfering directly with the SMC homeostatic stress state or with continuity of the force sensing apparatus. We also have preliminary data indicating that force promotes micro-remodeling of the elastin contractile unit. Therefore, defects in the mechanical environment (such as changes in the universal elastic modulus) or the cellular interpretation of the mechanical environment (such as defects in the elastin-contractile unit) lead to maladaptive remodeling associated with disease. Disruption of the physical and functional connecting elastin-contractile units weakens the aortic wall and exacerbates pathological conditions due to alterations in SM phenotype. Ineffective regeneration of elastic fibers hampers the control of these conditions and urges us to have a more comprehensive understanding of the regulation...
of elastic fibers and organization of elastin-contractile units in vivo.

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