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JACC FOCUS SEMINAR: EXTRACELLULAR MATRIX IN CARDIOVASCULAR HEALTH AND DISEASE

JACC FOCUS SEMINAR

Extracellular Matrix in Vascular Disease, Part 2/4

JACC Focus Seminar

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ABSTRACT

Medium-sized and large arteries consist of 3 layers: the tunica intima, tunica media, and tunica adventitia. The tunica media accounts for the bulk of the vessel wall and is the chief determinant of mechanical compliance. It is primarily composed of circumferentially arranged layers of vascular smooth muscle cells that are separated by concentrically arranged elastic lamellae; a form of extracellular matrix (ECM). The tunica media is separated from the tunica intima and tunica adventitia, the innermost and outermost layers, respectively, by the internal and external elastic laminae. This second part of a 4-part *JACC* Focus Seminar discusses the contributions of the ECM to vascular homeostasis and pathology. Advances in genetics and proteomics approaches have fostered significant progress in our understanding of vascular ECM. This review highlights the important role of the ECM in vascular disease and the prospect of translating these discoveries into clinical disease biomarkers and potential future therapies. (J Am Coll Cardiol 2020;75:2189–203)

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ABBREVIATIONS AND ACRONYMS

ADAMTS = a disintegrin and metalloproteinase with thrombospondin motifs

ADCL = autosomal dominant cutis laxa

BMP = bone morphogenetic protein

ECM = extracellular matrix

EDS = Ehlers-Danlos syndrome

ELN = elastin-encoding gene

GAG = glycosaminoglycan

MFAP = microfibrillar-associated protein

MFS = Marfan syndrome

MMPs = matrix metalloproteinases

PLOD = procollagen-lysine 2-oxoglutarate 5-dioxygenase

PWV = pulse wave velocity

SLRPs = small leucine-rich proteoglycans

TAA = thoracic aortic aneurysm

TGF = transforming growth factor

TIMPs = tissue inhibitors of metalloproteinases

vEDS = vascular Ehlers-Danlos syndrome

VSMCs = vascular smooth muscle cells

The first paper in this *JACC* Focus Seminar series already covered basic extracellular matrix (ECM) biology. Here, in part 2 of this 4-part series, we address the role of the ECM in aneurysm, arterial stiffening, atherosclerosis, and other vascular diseases.

The wall of medium-sized and large arteries, including the aorta, consists of 3 distinct tissue layers: the tunica intima, tunica media, and tunica adventitia. The tunica intima lines the luminal part of the blood vessel and encompasses a single layer of endothelial cells positioned on its own basement membrane and the proteoglycan-rich subendothelial space. The middle layer (i.e., the tunica media) is separated from the tunica intima by the internal elastic lamina. The tunica media accounts for the bulk of the vessel wall tissue and is the chief determinant of mechanical compliance. It is primarily composed of circumferentially arranged vascular smooth muscle cells (VSMCs), collagens, and elastin. VSMCs are separated by and adhere to concentrically arranged elastic lamellae interacting with other ECM components. This specific arrangement of cells and ECM has a major role in mechanosensing and force resistance, as emphasized by the concept of the "elastin-contractile unit" in vascular wall homeostasis (1). The tunica media is separated from the outermost arterial layer (i.e., the tunica adventitia) by the external elastic lamina. The tunica adventitia is composed of collagenous ECM, fibroblasts, perivascular nerves, lymphatic vessels, vasa vasorum, and inflammatory cells and has recently attracted attention as a vascular stem cell or progenitor cell niche (2,3). The exact composition of the tunica adventitia depends on arterial size and function.

HIGHLIGHTS

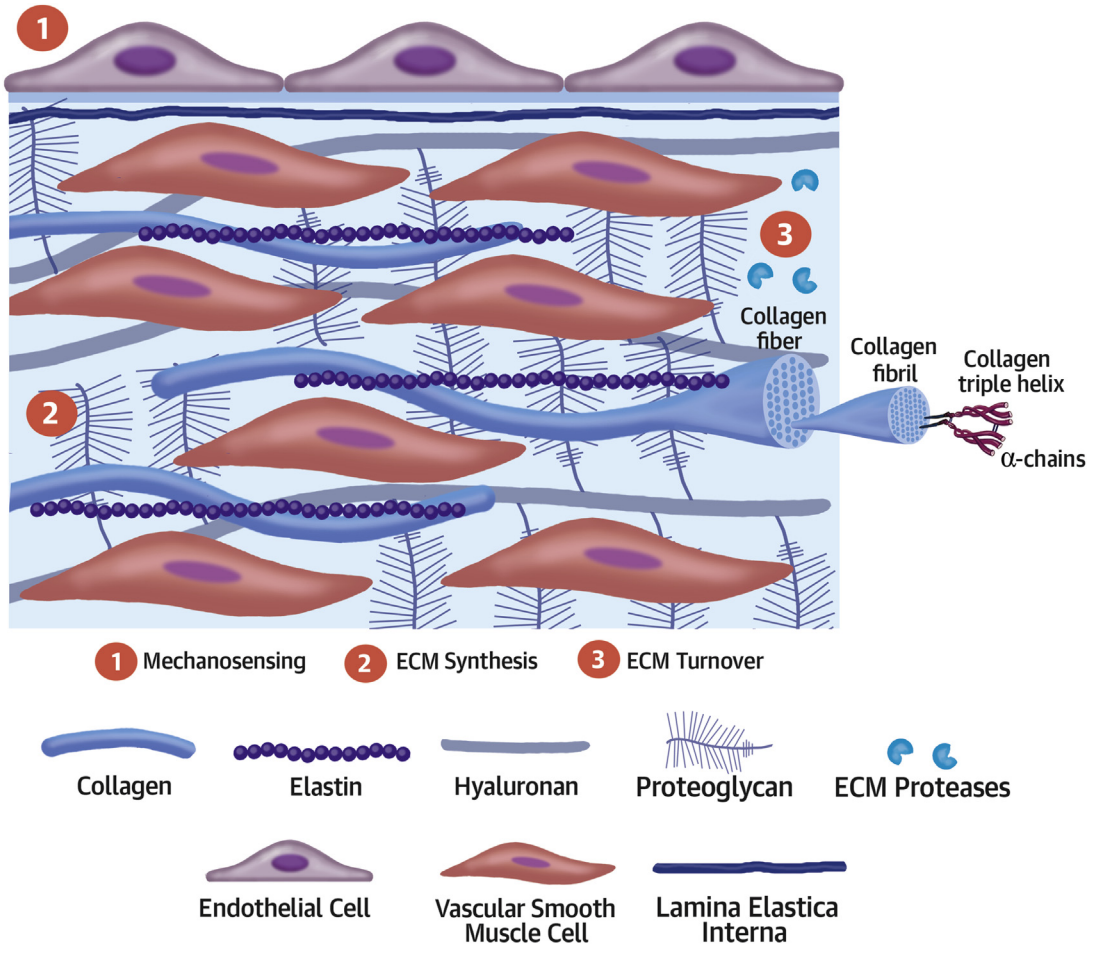
- The middle arterial layer, the tunica media, is composed of circumferentially arranged layers of VSMCs that are separated by concentrically arranged elastic lamellae, a form of ECM.
- Advances in genetics and proteomics approaches have facilitated dramatic progress in our understanding of the key role of the ECM in vascular health and disease.
- The emerging discoveries regarding the role of ECM in the vasculature hold promise to be developed into novel clinical disease biomarkers and even future therapies.

COMPOSITION OF THE VASCULAR EXTRACELLULAR MATRIX

VSMCs produce ECM that allows adaptions to mechanical forces that act on the vessel wall while maintaining adequate wall pressures (**Central Illustration**). Hence, the composition of this extracellular meshwork must be tailored to the mechanical demands of the vessel wall. The arterial wall holds both elastic and viscous properties that are needed for adequate blood pressure propagation (**Figure 1**) (4). This ensures continuous perfusion of peripheral capillaries. Although the core vascular ECM or matrisome comprises approximately 300 proteins (5–7), the essential mechanical or viscoelastic properties of the vessel wall (i.e., high resilience, low hysteresis, and nonlinear elasticity) are mostly provided by 3 main constituents: elastic fibers, fibrillar collagens, and large aggregating proteoglycans (**Central Illustration**).

ELASTIN. Elastin is the chief constituent of vascular elastic fibers and is formed by multimerization and cross-linking of its hydrophobic monomers, called tropoelastins. In the aorta, these molecules are predominantly produced by medial VSMCs. This process begins before birth and serves to counterbalance the mechanical forces acting on large conduit vessels (5), thus allowing for elastic recoil and therefore repeated stretch and relaxation cycles whereby tropoelastin monomers polymerize with each other through coacervation (the formation of polymer droplets based on the separation of 2 differing liquid phases) (6). This process is directed by the alternating hydrophobic and hydrophilic sequences in tropoelastin that determine intermolecular alignment, resulting in cross-linked arrays of tropoelastin. In mature elastic fibers, the central elastin core is surrounded by microfibrils, which consist of several proteins, most notably fibrillins and microfibril-associated glycoproteins (7). Interestingly, genetic deletion of elastin results in lethality shortly after birth as a consequence of aortic obstruction from VSMC proliferation (8). This overgrowth of VSMCs suggests that elastin normally suppresses VSMC proliferation. Heterozygous elastin-deficient mice are viable but develop hypertension and show decreased aortic compliance with thinner elastic lamellae, but more lamellar units (9). An

CENTRAL ILLUSTRATION **Key Elements of the Arterial Wall and Vascular Extracellular Matrix Components**



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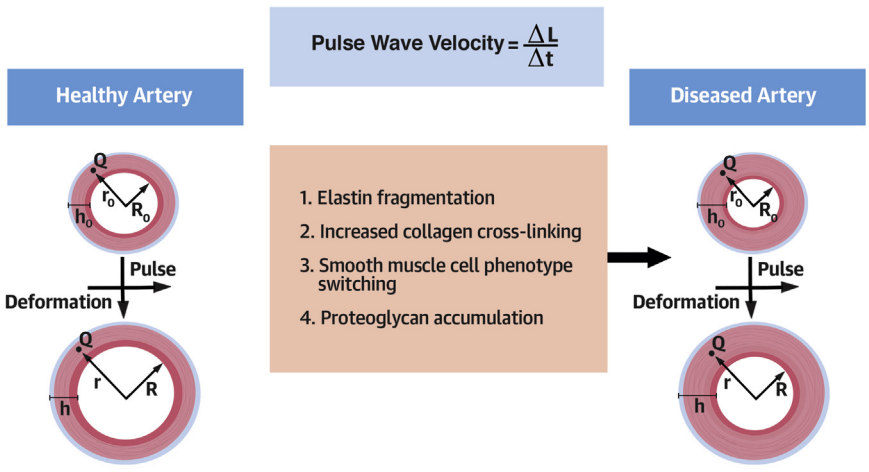
The mechanical or viscoelastic properties provided by the extracellular matrix (ECM) are determined by 3 main constituents: elastic fibers, fibrillar collagens, and large aggregating proteoglycans such as aggrecan and versican. Mechanosensing by both endothelial cells as well as vascular smooth muscle cells (VSMCs) and the highly connected ECM network in which they are embedded, is vital for vessel wall homeostasis. Both changes in viscoelasticity , i.e., as a consequence of aging, and changes in mechanical stress, i.e., as a consequence of hypertension, are accompanied by changes in the composition of the vascular ECM.

increase in arterial stiffness occurs before changes in blood pressure and arterial morphology (10). Indeed, the mechanical properties of arteries in the low blood pressure environments of some invertebrates rely almost entirely on microfibrils, whereas high blood pressure environments require more resilient proteins, such as an elastomer in the octopus (11) and elastin in most vertebrates (12). Therefore, in vertebrates, elastin deposition on microfibrils resulting in an elastic fiber network and elastic lamellae may have occurred with the development of a closed circulatory system providing the mechanical properties that enabled the higher pressures of pulsatile blood flow (12,13).

COLLAGENS. Connected to elastin are strong fibrillar collagens, arranged as long cross-linked fibers that provide arterial stiffness (referring to the force required to deform the vessel wall during systole) and vessel wall strength (the maximum force that can be applied to the vessel wall before its failure). Collagens form an intricate ECM meshwork with unique architectural compositions. Three individual polypeptide

FIGURE 1 Schematic Overview of the Impact of Altered ECM in Diseased Arteries



In terms of a general comparison between diseased versus healthy arteries, vascular smooth muscle cells (VSMCs) switch from a contractile to a synthetic phenotype and the accumulation of aggrecan/versican-type proteoglycans leads to a more hydrated extracellular matrix (ECM) to maintain vessel wall viscosity. Enhanced proteolysis results in fragmentation of elastin. Loss of elasticity or increased mechanical stress is compensated by enhanced collagen cross-linking contributing to arterial stiffness. Increased pulse wave velocity (PWV), the clinical measure of vascular stiffness, is reflected by an acceleration in pulse wave propagation; PWV = ΔL or the distance between position A and position B/Δt or the time it takes for the pulse wave to travel the distance between position A and position B. In healthy vasculature, in response to the pressure (ΔP) created by ventricular contraction (systole), viscoelastic arteries experience deformation followed by recoil (diastole) allowing pulse wave propagation. Deformation and recoil of the arterial wall depend on the dimensions of the arterial wall (ΔA), which are primarily determined by systolic and diastolic wall thickness (h_O, h), radius of the vessel lumen (R_O, R) as well as the radius of the lumen including the vessel wall (r_O, r) (4). Changes in pulse pressure result in changes in the dimensions of the arterial wall as a consequence of altered ECM synthesis and turnover.

alpha-chains are coiled around each other to form a triple helix. Besides this triple-helical domain, collagens contain non-triple-helical domains that are used as attachment sites by other ECM proteins. Collagens differ by the length of these amino acid repeat motifs, which allow the formation of supramolecular aggregates that can arrange into varying geometric networks. This structural heterogeneity allows for functional diversity. The supramolecular assembly is further directed by a combination of tissue-specific matrix macromolecules, such as fibronectin and proteoglycans, cell surface integrins, and intracellular forces. In total, there are 28 individual collagens: collagens range from thick parallel bundles or fibril-forming collagens (I, II, III, V, XI, XXIV, XXVII) and fibril-associated collagens with interrupted triple helices (FACITs) (IX, XII, XIV, XVI, XIX, XX, XXI, XXII) to network-forming collagens (IV, VIII, X), transmembrane collagens (XIII, XVII, XXIII, XXV), beaded filament collagens (VI, XXVI, XXVIII), and anchoring fibril-forming collagens (VII) (14,15).

The major collagens in large elastic arteries are the fibrillar collagens (types I, III, V), collagen IV, and collagen VI (16). Specifically, in the aorta collagens type I and III constitute the largest portion. Although type III is more abundant in the medial layer, type I is the predominant form in the adventitia. In murine models, mutations in collagen type I, III, and V result in premature mortality as a consequence of vascular rupture (17-19). Fibrillar collagens are enzymatically cross-linked during deposition by lysyl oxidase and lysyl oxidase-like proteins (providing strength), and they are non-enzymatically cross-linked under pathological conditions such as oxidative stress resulting from hyperglycemia by advanced glycation end products (increasing stiffness) (20,21). The inhibition of collagen cross-linking in mice led to accelerated aneurysm formation in response to blood pressure elevation by angiotensin II administration, a finding highlighting the importance of cross-links in collagen fiber maturation for providing strength to the arterial wall (22). Small leucine-rich proteoglycans (SLRPs) support collagen fibrillogenesis (20). SLRPs (i.e., decorin, fibromodulin, and lumican) contain collagen-binding domains that allow for the formation of a protein coat on the surface of the fibrils, thus supporting collagen fibril formation and

protection of collagen fibrils from protease degradation (23). Furthermore, the interaction of fibromodulin with collagen cross-linking sites activates lysyl oxidase-like proteins and enhances collagen cross-linking (24). Knockout models for SLRPs confirm aberrant collagen fibrillogenesis creating ECM fragility (25,26). Concordantly, a negative correlation of SLRPs with the medial elastic modulus of the arterial wall was observed in the internal mammary artery of patients with high pulse wave velocity (PWV) (27).

Network-forming (collagen IV) and beaded filament (collagen VI) collagens are located in the basement membrane, as well as in the incomplete basement membrane layer surrounding VSMCs. Mice that are homozygous for a null allele of the *Col4a1/2* locus showed lethality between embryonic days 10.5 and 11.5 (28). These structural deficiencies in the basement membranes suggest that collagen IV is fundamental for the maintenance of integrity and function of basement membranes under conditions of increasing mechanical demand (28). Furthermore, mutations in *COL4A1* are known to be associated with adult small vessel disease, familial porencephaly, and HANAC (hereditary angiopathy with nephropathy, aneurysms, and muscle cramps) syndrome (29). Mice with a homozygous missense mutation for *Col4a1* showed focal detachment of the endothelium from the tunica media (30). Interestingly, basement membrane thickening is among the hallmarks of early diabetic microangiopathy as a consequence of hyperglycemia (31,32). Beaded filament collagen VI is also critically involved in basal lamina assembly, an important aspect of vessel development. Collagen VI is found in association with fibrillin-1 in the media of the human aortae connecting elastic laminae to the basement membrane of VSMCs (16). Absence of collagen VI in mice reduced vascular basal lamina assembly and increased vessel leakage (33).

PROTEOGLYCANS. Whereas collagens provide tensile strength that enables resistance to deformation and preserves vessel wall integrity, elastin allows for distensibility and reversible recoil, thus enabling tissues to withstand repeated expansion. Importantly, in addition to collagens and elastin, proteoglycans also contribute to the mechanical properties of the vessel wall. Proteoglycans consist of protein cores that are decorated with glycosaminoglycan (GAG) side chains. A comprehensive description of proteoglycans can be found elsewhere (34). With regard to the vasculature, SLRPs not only are important for collagen formation but also contribute to the maintenance of growth factor stores (35). Proteoglycans localized at the basement membranes (i.e., agrin, perlecan, and collagens XV and XVII) or associated with cell membranes (i.e., dystroglycan and glypicans) typically have large protein cores and contain GAG side chains with heparan sulfate. Of special interest are large aggregating proteoglycans such as versican and aggrecan, which have protein cores decorated with dozens of GAG side chains.

In an elastic artery, the arterial wall retains part of the blood pressure-induced deformation, which causes energy dissipation. This energy loss is recovered elastically, a process known as hysteresis, and indicates high vascular resilience (36). The time delay in the recoil of the vessel wall on unloading ensures lower pulse pressures and adequate pulse wave propagation. This property of the vessel wall is, to a large extent, provided by hyaluronan-proteoglycan aggregates (i.e., large aggregating proteoglycans) and is known as wall viscosity. Arterial viscosity, however, has not been evaluated as comprehensively as other mechanical properties of arteries such as compliance, distensibility, and stiffness (37). Increased wall viscosity, as measured using tonometry and an automatic densitometric analysis incorporating pressure and diameter loops, showed that intima-media thickening as observed in hypertensive patients is related to ECM-VSMC alterations represented by increased vascular viscosity (38). Another study showed that central artery vessel wall viscosity increases with advancing age (39). Although the role of large aggregating proteoglycans is not yet fully understood, their negatively charged GAG side chains create a hydrated environment while simultaneously retaining lipoproteins, growth factors, and cytokines (40,41). Ex vivo removal of GAGs from the porcine thoracic aorta led to a decrease in stress-relaxation of the vessel wall, a finding suggesting that the interaction between GAGs and other ECM components is important for normal arterial wall mechanics (42). Versican, the main large aggregating proteoglycan in the vessel wall, is known to increase after vascular injury and accumulates in advanced atherosclerotic plaques (43–46).

The main proteases responsible for large aggregating proteoglycan turnover are disintegrin and metalloproteinase with thrombospondin motifs (ADAMTSs) (47). The balance between clearance and retention of ADAMTSs is critical in maintaining vascular integrity, and this balance is regulated by cell surface receptors, including the low-density lipoprotein receptor-related protein 1 (LRP1) (48). ADAMTSs are increasingly recognized as important regulators of large aggregating proteoglycan turnover in atherosclerosis and aneurysm formation. For

example, in a mouse model of angiotensin II infusion, lack of ADAMTS-5 activity contributes to increased aortic dilatation and higher blood pressure (49). ADAMTS-1 haploinsufficiency causes thoracic aortic aneurysm and dissection in mice (50). Similarly, versican and aggrecan, 2 highly homologous large aggregating proteoglycans, accumulate in patients with thoracic aortic aneurysms and dissections (51). Moreover, increased expression of ADAMTS-4 and accumulation of versican fragments were observed during the earliest phase of cerebral cavernous malformations (52). In addition to the different members of the ADAMTS family, the role of bioactive versican fragments generated by ADAMTS-mediated cleavage remains to be explored with regard to vascular disease (53).

EXTRACELLULAR MATRIX REMODELING, ARTERIAL STIFFNESS, AND ATHEROSCLEROSIS

Vascular remodeling first occurs during embryonic development, when shear stress is important for the configuration of large arteries (54). Although flow supports vascular development, shear stress alone is sufficient to induce vascular remodeling (55). Furthermore, recruitment of VSMCs to the remodeled capillary network during embryonic development is essential for the development of large arteries. This is regulated by Kruppel-like factor 2, a transcription factor regulating several mechanosensitive genes. Aortas from Kruppel-like factor 2–null mice lack an organized, compact tunica media and show a significant reduction in the number of differentiated VSMCs, as well as a decrease in ECM deposition (56). ECM degradation and remodeling are important hallmarks of major vascular diseases, including hypertension and arterial stiffness, arteriosclerosis, aneurysm formation, and atherosclerosis, which are further discussed here.

HYPERTENSION AND ARTERIAL STIFFNESS. Arterial stiffness is one of the earliest clinically detectable manifestations of adverse vascular remodeling (**Figure 1**). Degenerative stiffness of the arterial wall, which is associated with hypertension, is also referred to as arteriosclerosis, or "hardening of the arteries." Arteriosclerosis is not to be confused with atherosclerosis, although both coexist and refer to progressive, diffuse, and age-related processes (57). The Doppler echocardiographic method of PWV determination provides a reproducible measurement of elastic properties (58) (**Figure 1**). Indeed, arterial stiffness as measured by PWV is an independent predictor of coronary heart disease and stroke (59). In a proteomics study by Lyck Hansen et al. (60), the vascular content of SLRPs in mammary arteries was found to be associated with PWV. In fact, collagen metabolism is thought to be important for the pathogenesis of arterial stiffness and remodeling in hypertension. For example, collagen abundance is increased in mesenteric small arteries of spontaneously hypertensive rats (61) and subcutaneous resistance arteries in patients with essential hypertension (62). Although genome-wide association studies have identified only a small number of genetic variants reaching genome-wide significance, the *COL4A1* gene polymorphism on chromosome 13 (collagen type 4) was associated with higher arterial stiffness (63) in 1 study, but the association failed to be replicated in a more recent meta-analysis (64). As an additional factor, fragmentation of load-bearing elastin fibers secondary to fatigue and pulsatile tensile stress contributes to arterial stiffness (65). In patients with hypertension, the increase in mechanical load on the vasculature leads to changes in collagen abundance and cross-linking, which are among the first structure-function alterations that occur with this disease (65,66) (**Figure 1**).

Remodeling of the arterial wall is primarily induced by collagenases or metalloproteinases (MMPs), a family of zinc-dependent endopeptidases (67,68). An early study by Pollanen et al. (69) found that a polymorphism in the promoter of MMP9 leading to higher expression of the protease was associated with greater stiffness in large arteries, secondary to excessive MMP9 activity. The same investigators found a polymorphism in MMP3 associated with decreased stiffness in the older population (70). Other studies found a positive correlation between PWV and the ratio of tissue inhibitors of metalloproteinases (TIMPs) to MMPs (71,72), as well as increased procollagen expression (66), findings suggesting a compensatory response to increased ECM degradation.

Microscopically, stiffened vessels show increased collagen deposition and MMP expression, fragmented and diminished elastin, disorganized endothelium, and infiltration of macrophages and mononuclear cells (73) (**Central Illustration**). Calcium deposition in the arterial wall also increases with age and is characterized by the direct binding of calcium ions to elastin fibers (74). Animal models of elastocalcinosis reveal that increased elastin fragmentation in the tunica media is associated with arterial stiffness (75,76). Furthermore, aging increases the proportion of senescent cells within the vasculature, thereby inducing a state of chronic inflammation (77). This inflammation, combined with reduced expression

levels of TIMPs, reduced cross-linked elastin content, and elevated levels of activated MMPs and other proteases, collectively compromises the integrity of the elastin-collagen networks and the basement membrane (78). The compensatory increase in collagen production results in poorly organized and highly cross-linked (stiffer) collagens (79,80).

ATHEROSCLEROSIS. Heritable factors (i.e., "genetics") play a major causal role in atherosclerosis and coronary artery disease (81). Accordingly, genome-wide association studies such as the transatlantic Coronary ARtery DIsease Genome wide Replication and Meta-analysis (CARDIoGRAM) consortium (82), identified relationships between genetic variations and coronary artery disease (83). Among the risk loci were ECM and basement membrane genes, including collagens IV (*COL4A1/COL4A2*), integrin subunit beta 5 (*ITGB5*), and fibronectin (*FN1*). Interestingly, the antiapoptotic effect of cell anchoring to the ECM by ITGB5-FN1 interactions (84) underlines the importance of cell-ECM connectivity in vascular disease. A *COL4A2* variant was associated with both *COL4A1* and *COL4A2* expression, as well as VSMC survival, ECM remodeling, and atherosclerotic plaque stability (85). Therefore, alterations in basement membrane proteins (86) may influence key cellular events in coronary artery disease development. Furthermore, genetic mutations affecting MMPs have been associated with arterial stiffness and atherosclerotic lesion progression (69,70). For example, the finding that *Mmp8* knockout mice (on an *ApoE*$^{-/-}$ atherosclerosis-prone background) have atherosclerotic lesions with fewer macrophages but increased collagen content was also validated in humans in the Bruneck cohort, where an association was found between *MMP8* gene variation and the extent of coronary atherosclerosis in patients with coronary artery disease. Interestingly, the polymorphism found in these patients involves the propeptide of MMP8, thus making it less amenable to protein activation (87). Further studies are required to understand the mechanisms by which other ECM proteases affect the progression of atherosclerosis.

According to the response-to-retention hypothesis, the binding of cholesterol-containing lipoprotein particles to intimal proteoglycans is the central pathogenic process in atherogenesis. Once retained, lipoproteins become oxidized, accumulate in "foam cells," and provoke a cascade of inflammatory processes that drive the atherosclerotic process and that ultimately define the propensity to plaque rupture (88). Glycosylation is important for proteoglycan function, as exemplified by the chemokine and growth factor–binding properties of GAG side chains. In particular, versican and biglycan retain low-density lipoproteins (89,90), thereby resulting in lipid deposition in the subintimal space leading to atherosclerosis. Accumulation of versican, the main large aggregating proteoglycan in normal arteries, is seen in early stages of atherosclerosis and predisposes to lipoprotein retention (91). Furthermore, versican accumulates in response to aging, hypertension, and injury (92). Thus, proatherogenic stimuli affecting versican expression or its GAG composition may contribute to enhanced plaque development (92). Biglycan is also implicated in the retention of lipoproteins. However, contrary to expectations, biglycan deficiency accelerated rather than reduced atherosclerosis in *ApoE*$^{-/-}$ mice (93). Notably, increased lesion formation in the aortas of *ApoE*$^{-/-}$*Biglycan*$^{-/-}$ mice was associated with a marked increase of the large aggregating proteoglycan aggrecan.

Although versican is the main hyaluronan-binding proteoglycan in the arterial wall, aggrecan, found in articular cartilage, is predominantly induced in the vessel wall on injury. In cartilage, negatively charged GAGs on the surface of aggrecan attract water and therefore confer resistance to compression. Aggrecan was first identified in human vessels by proteomics studies (94). In a porcine model of coronary stenting, aggrecan expression increased in response to stent implantation (40). On the basis of proteomics data from the human vasculature, aggrecan expression is low in veins compared with arteries (40,95,96). In a mouse model of vein graft disease, however, aggrecan was induced in veins grafted to the arterial circulation (40). In contrast, loss of aggrecan is observed in human varicose veins with their characteristic dilation and tortuous appearance (97). Finally, the importance of proteoglycan glycosylation was illustrated by the correlation of age-related aortic stiffening with a loss of GAG (chondroitin sulfate)-binding domains of aggrecan with age (98). The sulfation pattern of these GAGs further emphasize the importance of large aggregating proteoglycans in vascular disease (46,99). Collectively, these data demonstrate the major role that ECM and ECM-related proteins play in the development and progression of atherosclerotic disease.

EXTRACELLULAR MATRIX AND AORTIC ANEURYSM

Aortic aneurysms result from progressive enlargement or bulging of the aorta secondary to wall weakness. Abdominal aortic aneurysms (located below the diaphragm) are far more prevalent than their thoracic

counterparts (thoracic aortic aneurysms [TAAs], located above the diaphragm). TAAs have been more extensively studied, however, because they affect younger individuals and have a higher heritability. In 20% to 25% of patients with TAAs, a positive family history is apparent, and in about one-third of probands a monogenic cause can be identified with current sequencing technologies (100). Connective tissue disorders with mendelian inheritance (e.g., autosomal dominant or recessive) manifesting with aortic aneurysm represent an important paradigm for the study of the pathogenesis of TAA in general.

PATHOMECHANISMS OF AORTIC ANEURYSM: FROM MEDIAL DEGENERATION TO MATRIX-INDUCED CYTOKINE SIGNALING DEFECTS. Aortic lesions from patients with TAAs are histopathologically characterized by (cystic) medial degeneration, manifesting with elastic fiber fragmentation, collagen fiber disorganization, and proteoglycan accumulation, as well as VSMC loss and contractile-to-synthetic phenotype switching of these cells (101). VSMCs are considered the protagonists in TAA development. In addition to being less contractile and more prone to apoptosis, they make a major contribution to the ECM abnormalities that lead to medial degeneration. In normal aortas, VSMCs maintain ECM homeostasis by secreting proteases (MMPs) and inhibitors (TIMPs) in a tightly controlled manner. In the arterial wall of TAA, however, excessive MMP secretion occurs, leading to disproportionate ECM degradation (102,103). This prominent ECM deterioration leads to the release of cytokines involved in signaling pathways that regulate ECM homeostasis (e.g., transforming growth factor [TGF]-β signaling or bone morphogenetic protein [BMP] signaling) and whose bioavailability is strictly controlled by ECM sequestration (**Figure 2**) (104,105). Collectively, these pathological events lead to weakening of the aortic wall, thus diminishing its ability to withstand the biomechanical forces imposed by pulsatile blood flow and blood pressure.

ANEURYSM DISORDERS SECONDARY TO STRUCTURAL EXTRACELLULAR MATRIX DEFICIENCY.

Altered collagen is an important cause of aortic aneurysm. Over the past decades, genetic defects in several collagen-encoding genes (i.e., *COL1A1*, *COL1A2*, *COL3A1,* and *COL5A1*) have been linked to aortic aneurysm development. Most prominently, mutations in *COL3A1* can be found in more than 95% of patients with vascular Ehlers-Danlos syndrome (vEDS) (106), which is an autosomal dominant connective tissue disorder characterized by skin translucency and easy bruising, as well as artery and internal organ fragility. This commonly leads to life-threatening complications such as arterial aneurysms and/or ruptures, as well as gastrointestinal perforations and joint laxity primarily affecting the small hand joints. In vEDS, glycine-affecting missense mutations are identified in the majority of patients, but splice site mutations and (multi-)exon deletions have also been described. Typically, *COL3A1* mutations resulting in haploinsufficiency are associated with a milder phenotype (107).

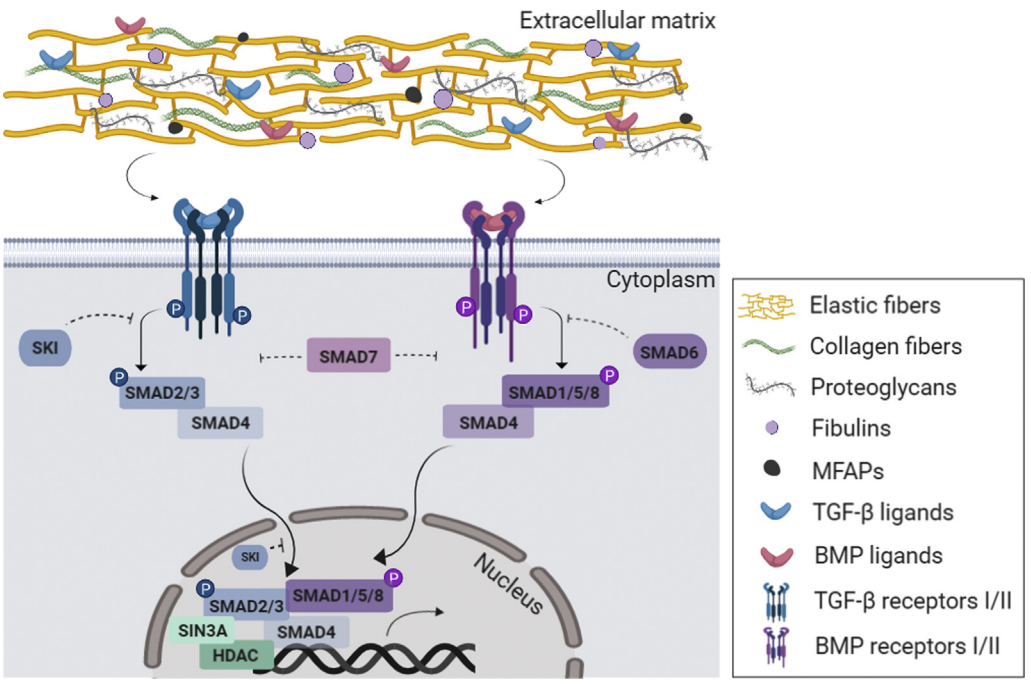
Mutations in *COL5A1*, which normally cause classical Ehlers-Danlos syndrome (EDS) and typically lead to joint hyperextensibility and atrophic scars, have occasionally been found in patients with a clinical diagnosis of vEDS (108–111).

Collagen V serves as a cross-linker and regulator of the diameter of collagen I fibers. Mutations in *COL1A1* and *COL1A2* are a common cause of osteogenesis imperfecta (112), a connective tissue disorder that is most prominently characterized by multiple fractures, skeletal deformity and/or short stature, dentinogenesis imperfecta, hearing loss, and blue sclerae. Aortic aneurysms or dissections occur in 10% to 30% of patients with osteogenesis imperfecta (113–115), and *COL1A1* or *COL1A2* mutations have been found in some of these patients (116).

The role of elastin in aortic aneurysm. Dominant-negative mutations in the elastin-encoding gene (*ELN*) cause autosomal dominant cutis laxa (ADCL), characterized by inelastic and sagging skin (117). Truncating *ELN* mutations, conversely, cause supravalvular aortic stenosis (118). TAA has been described in 30% to 50% of patients with ADCL (119,120). *ELN*-related aortopathy in patients with ADCL can range from mild dilatation to aortic rupture, even within the same family (121). Remarkably, a triplication encompassing the *ELN* gene has been found to segregate with disease in a TAA-affected family (122). It is hypothesized that gain-of-function of elastin contributes to TAA risk, but it remains unclear why these patients do not have cutis laxa (which is also associated with gain-of-function point mutations in *ELN*).

Fibrillin and aortic disease. The cysteine-rich glycoproteins fibrillin-1 and fibrillin-2 are the main structural elements of the microfibril scaffold (i.e., another major component of elastic fibers). Besides elastin, homopolymerized and heteropolymerized fibrillins bind integrins, proteoglycans, and latent TGF-β, among other ECM-resident proteins. Dominant mutations in the fibrillin-1 (*FBN1*) and fibrillin-2 (*FBN2*) genes cause Marfan syndrome (MFS) and congenital contractural arachnodactyly (CCA), respectively (123,124). MFS is a multisystem disorder manifesting with a pleiotropy of skeletal (e.g., bone

FIGURE 2 **Simplified Schematic Overview of the Link Between ECM Components and the TGF-β and BMP Signaling Pathways**



The extracellular matrix (ECM) provides structural support to our body's organs and sequesters growth factors. With loss of extracellular matrix integrity and strength, tissue weakness may occur, as well as uncontrolled release of growth factors. BMP = bone morphogenetic protein; HDAC = histone deacetylase; MFAPs = microfibrillar-associated proteins; P = phosphorylated; TGF = transforming growth factor.

overgrowth, joint laxity), ocular (e.g., ectopia lentis), skin (e.g., striae), and cardiovascular manifestations, including TAAs leading to life-threatening aortic dissections. Approximately 1,850 different *FBN1* mutations have been described to date, including nonsense, frameshift, splice altering, insertion/deletion, and missense variations, as well as whole-gene or multiexon deletions. Congenital contractural arachnodactyly manifests with joint contractures, arachnodactyly, crumpled ears, and kyphoscoliosis as main features. Although significantly fewer *FBN2* mutations have been reported, the mutation spectrum appears similar to that of *FBN1*. Aneurysmal disease is a rather uncommon manifestation in *FBN2* patients. However, carriers of *FBN2* mutations resulting in exon 32 skipping have been suggested to be at significant risk for progressive aortopathy necessitating serial cardiovascular imaging in this specific subset of patients (125).

Other matrix proteins and thoracic aortic aneurysm. TAA-causing mutations have also been found in members of the proteoglycan, fibulin, and microfibrillar-associated protein (MFAP) families. Proteoglycans fill much of the extracellular interstitial space in the form of a hydrated gel. In TAA medial degeneration lesions, mostly aggrecan and versican accumulate in the VSMC voids, thereby increasing intralamellar swelling pressure, disrupting cell-ECM interactions, and disorganizing the aortic wall's microstructure. Their massive deposition is attributed to increased aggrecan and versican mRNA expression, as well as a concomitant reduction in proteoglycan proteolysis by proteases of the ADAMTS family (51). The small leucine-rich proteoglycan biglycan, encoded by the X-linked *BGN* gene, is currently the only proteoglycan in which TAA-causing mutations have been identified. More precisely, loss-of-function mutations in *BGN* cause Meester-Loeys syndrome (i.e., a severe TAA syndrome characterized by hypertelorism, pectus deformity, joint hypermobility, contractures, and mild skeletal dysplasia) (126).

Fibulins are evolutionary highly conserved ECM proteins that are associated with basement membranes and elastic fibers. Of all fibulins that have been reported to contribute to elastogenesis to date, fibulin-4 and fibulin-5 are the most critical and nonredundant. More specifically, they potentiate the efficiency and maturation of tropoelastin multimerization, facilitate elastin cross-linking, and assist

elastin deposition onto microfibrils (127,128). Recessive mutations in their respective genes (i.e., *FBLN4* and *FBLN5*) cause cutis laxa syndromes. Whereas *FBLN5* mutation carriers often present with lung emphysema, *FBLN4* deficiency is tightly linked with aortic or arterial aneurysms and tortuosity (129,130). Although fibulin-5 is expressed at much higher levels than fibulin-4 in the human aorta, aortopathy is present only in *FBLN4* mutation carriers. Investigators have postulated that this phenomenon arises because fibulin-4 also plays a nonredundant role in maintaining collagen integrity in the vessel wall, whereas fibulin-5 does not (131).

Finally, dominant TAA-causing loss-of-function mutations have been identified in microfibrillar-associated protein 5 (*MFAP5*) (132). *MFAP5* localizes to microfibrils and can reversibly bind growth factors, including TGF-β and BMP. Only a few families have been reported, thus impeding comprehensive delineation of the *MFAP5*-associated phenotype. However, it appears to be nonsyndromic.

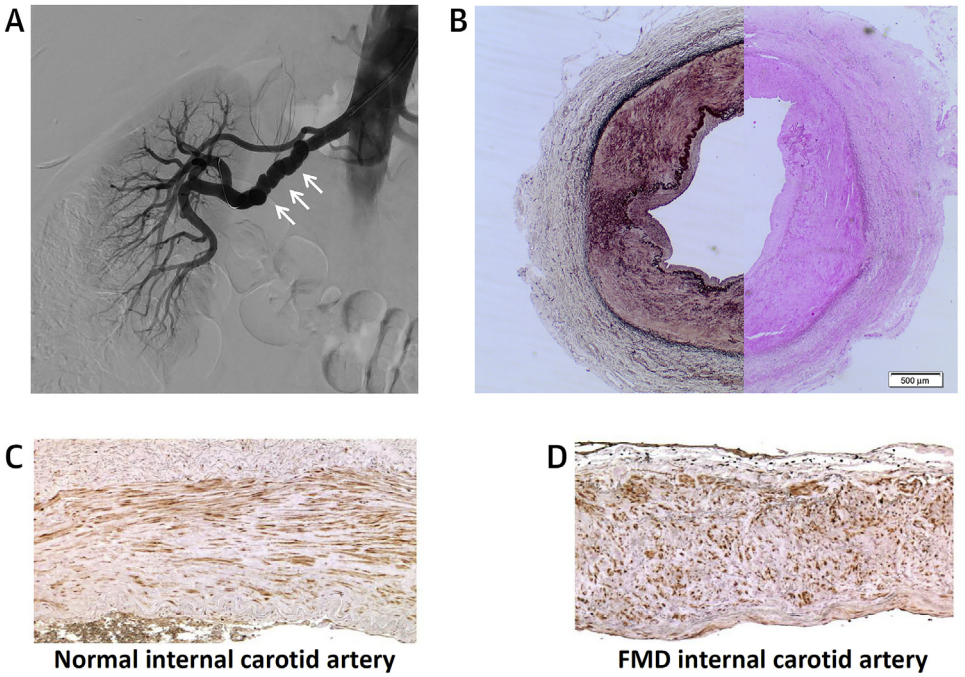
Matrix-modifying enzymes and aortic aneurysm. Elastin and collagen cross-linking are mediated by a tightly regulated enzymatic process encompassing modification of specific collagen and elastin amino acid residues. Procollagen-lysine 2-oxoglutarate 5-dioxygenase (PLOD) enzymes catalyze hydroxylation of lysine before collagen secretion. Lysyl oxidases are copper enzymes that, on collagen secretion, take part in the initiation of elastin and collagen cross-linking by converting (hydroxyl)lysine residues to their respective aldehydes. Mutations in both types of enzymes have been linked to TAA development. Biallelic mutations in *PLOD1* cause kyphoscoliotic EDS, which is characterized by hypotonia, early-onset kyphoscoliosis, generalized joint hypermobility, skin fragility, and ocular manifestations (133). Aneurysms and dissections of the aorta and medium-sized arteries may occur in *PLOD1*-deficient patients. *LOX* encodes the most abundant lysyl oxidase in the aorta. Heterozygous mutations that disrupt its catalytic activity or lead to haploinsufficiency cause TAA, often in conjunction with other connective tissue manifestations (134).

ANEURYSM DISORDERS SECONDARY TO CYTOKINE SIGNALING DYSREGULATION. Transforming growth factor-β signaling. Pathologically increased TGF-β signaling was first implicated in the pathogenesis of TAA in the context of MFS (135). In mice with MFS, researchers showed that TAA development occurs in the context of TGF-β signaling dysregulation and that administration of TGF-β-neutralizing antibodies rescues the aneurysmal phenotype. Under normal physiological conditions, fibrillin-1-containing microfibrils strictly control ECM targeting of TGF-β ligands. Mutations in *FBN1* result in weakening of the microfibrillar network and hence lead to an unrestrained release of TGF-β ligands, disproportionately activating downstream TGF-β signaling. Interestingly, increased TGF-β signaling has also been seen in aortic walls of TAA-affected patients with mutations in genes coding for other ECM components (i.e., in *BGN* and *FBLN4*) (126,136).

Further establishment of a role for dysregulated TGF-β signaling in syndromic TAA came with the identification of loss-of-function mutations in TGF-β receptors (*TGFBR1* and *TGFBR2*), ligands (*TGFB2* and *TGFB3*), and downstream effectors (*SMAD2* and *SMAD3*) in patients with Loeys-Dietz syndrome, a TAA syndrome that has significant phenotypic overlap with MFS (**Figure 2**) (137). Whereas one would predict that loss-of-function mutations in key components of the TGF-β pathway would result in decreased TGF-β signaling, immunohistochemistry of aortic wall tissue of Loeys-Dietz syndrome patients revealed paradoxical up-regulation of TGF-β signaling. Mechanisms that could explain this remarkable observation are under investigation (138,139). Current evidence suggests an important role for disparate sensitivity of aortic VSMCs from different embryonic origins to overactive TGF-β (139). The identification of mutations in *SKI*, coding for a negative regulator of the TGF-β pathway (**Figure 2**), in another clinically similar TAA syndrome, Shprintzen-Goldberg syndrome, corroborated the conclusion that increased TGF-β signaling is an important mechanism contributing to syndromic TAA (140,141). Finally, dominant mutations in the juvenile polyposis syndrome and/or hereditary hemorrhagic telangiectasia gene *SMAD4* have also been associated with TAA (142). Notably, *SMAD4* encodes the only SMAD protein functioning in both the TGF-β and BMP signaling pathways.

Bone morphogenetic protein signaling. Similar to TGF-β ligands, bioavailability of BMP cytokines has been proposed to be regulated by microfibril-mediated sequestration in the ECM. Whereas interesting insights have been gained with respect to the effect of deficiency of ECM proteins implicated in syndromic TAA on BMP signaling in the musculoskeletal system (143,144), the respective effects in aortic tissue have (to our knowledge) not been investigated. With the recent identification of loss-of-function mutations in the BMP signaling inhibitor *SMAD6* in bicuspid aortic valve–related TAA (**Figure 2**) (145), and because TAA manifests in approximately 40% of *SMAD4* mutation carriers (142), BMP signaling has also been implicated in TAA, thus warranting this pathway's examination in other TAA presentations.

FIGURE 3 Role of ECM in Fibromuscular Dysplasia



(A) Catheter-based angiographic image representative of the typical appearance of multifocal fibromuscular dysplasia (FMD) affecting the renal artery, with a so-called string-of-beads appearance **(arrows)** that is classic for this disease. **(B)** Cross section of an artery affected by fibromuscular dysplasia (medial fibroplasia). Composite image with elastin staining on the **left,** and hematoxylin staining on the **right.** Note the dense fibrous connective tissue in the outer aspect of the media, disordered vascular smooth muscle cell arrangement, and collagen deposition. **(C and D)** Immunostaining of normal and fibromuscular dysplasia–affected internal carotid arteries by using anti-PHACTR1 antibody (phosphatase and actin regulator 1). *PHACTR1* was detected in endothelium and medial vascular smooth muscle cells. These images highlight the disordered architecture and disordered vascular smooth muscle cell arrangement of vessels affected by fibromuscular dysplasia. A single nucleotide polymorphism in *PHACTR1* was identified as the first fibromuscular dysplasia–associated genetic susceptibility locus (155). **(A)** Reproduced with permission from Olin et al. (153). **(B)** Reproduced with permission from Gottsater and Lindblad (154). **(C and D)** Reproduced with permission from Kiando et al. (155). ECM = extracellular matrix.

PROTEOMICS OF THE VASCULAR EXTRACELLULAR MATRIX

A limitation of the current approaches to study ECM is a focus on investigating individual factors in the pathophysiology whose biological functions are partially understood. Considering the connectivity of the intricate ECM protein network, genetic mutations in individual components will induce secondary remodeling processes that may impede the mechanical properties of the vasculature. Just knowing the causal mutations of particular ECM proteins does not provide insights into downstream ECM remodeling that leads to pathological alterations of the vessel wall.

Over the last few years, novel proteomics methods were introduced for a more systematic analysis of extracellular proteins (i.e., a proteomics method to characterize the vascular ECM) (95). This method, based on decellularization, and adaptions of it, have been extensively used by different groups to study vascular remodeling (49,51,146). Other ECM proteomics approaches include an activity-based proteomics approach to relate the activity of specific proteases to ECM degradation products and to identify novel protease targets (147), as well as workflows for the analysis of secreted proteins from VSMCs and endothelial cells (148,149). Currently, the latest glycoproteomics technologies allow an investigation of the involvement of glycosylation changes of ECM proteins in vascular disease (150), an unexplored area of vascular biology.

As an example of these complex networks of protein interactions, a glycoproteomics study of aortas from patients with MFS revealed an increase in MFAP4 as well as a different N-glycosylation pattern compared with control aneurysmal patients. Fibrillin-1 is essential for appropriate elastic fiber formation, but it also sequesters latent TGF-β. It has been proposed that

mutations in fibrillin-1 allow TGF-β levels to rise as a result of inadequate binding (151). TGF-β induces the expression of MFAP4 in both human and mouse VSMCs. An up-regulation of MFAP4 was also observed in aortic specimens from patients with a predisposition to TAA, such as patients with bicuspid aortic valves, when compared with carefully matched control aortic tissue. High plasma MFAP4 concentrations in patients with MFS were associated with a lower thoracic descending aorta distensibility and greater incidence of type B aortic dissection (150).

Thus, proteomics studies reveal the differential abundance of ECM proteins but also allow comparisons of their post-translational modifications (152). Such comprehensive studies can form the basis for further investigations exploring the role of ECM proteins in vascular disease.

SUMMARY AND CONCLUSIONS

The ECM is a critical component of the vessel wall. Changes in ECM proteins are ubiquitous across the complete spectrum of vascular disease, ranging from hypertension to aneurysm, to atherosclerosis, and even to rare vascular diseases. In many instances, these changes have been shown to be the inciting causal events underlying the associated vascular disease. Indeed, the data presented here in no way represent the full spectrum or importance of the ECM in vascular diseases. For example, the vascular disease fibromuscular dysplasia (**Figures 3A to 3D**) (153-155), which predominantly affects middle-aged women and is also linked to spontaneous coronary artery dissection (156), is likely to be associated with marked changes in the composition of the ECM in medium-sized and large arteries (157). However, although recent inroads have been made in understanding the genetics of fibromuscular dysplasia (153,155), we are far from properly understanding the nature of the changes that occur in the ECM as a result of this disease. This and many other knowledge gaps represent critical but exciting areas for further research.

Advances in genetics and proteomics approaches have fostered significant recent progress in our understanding of the essential role of the ECM in the vascular system. Ample evidence for a key role of altered ECM homeostasis and dysregulated matrix-induced cytokine signaling in TAA, hypertension, atherosclerosis, and other diseases has now been obtained. Although much remains to be accomplished, it is expected that in the coming years this increased knowledge will translated into novel disease biomarkers and potentially even into novel therapeutic approaches to treat these diseases (158).

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