



Published in final edited form as:

Circ Res. 2015 April 10; 116(8): 1448–1461. doi:10.1161/CIRCRESAHA.114.304936.

Role of Mechanotransduction in Vascular Biology: Focus on Thoracic Aortic Aneurysms and Dissections

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Abstract

Thoracic aortic diseases that involve progressive enlargement, acute dissection, or rupture are influenced by the hemodynamic loads and mechanical properties of the wall. We have only limited understanding, however, of the mechanobiological processes that lead to these potentially lethal conditions. Homeostasis requires that intramural cells sense their local chemo-mechanical environment and establish, maintain, remodel, or repair the extracellular matrix to provide suitable compliance and yet sufficient strength. Proper sensing, in turn, necessitates both receptors that connect the extracellular matrix to intracellular actomyosin filaments and signaling molecules that transmit the related information to the nucleus. Thoracic aortic aneurysms and dissections are associated with poorly controlled hypertension and mutations in genes for extracellular matrix constituents, membrane receptors, contractile proteins, and associated signaling molecules. This grouping of factors suggests that these thoracic diseases result, in part, from dysfunctional mechanosensing and mechanoregulation of the extracellular matrix by the intramural cells, which leads to a compromised structural integrity of the wall. Thus, improved understanding of the mechanobiology of aortic cells could lead to new therapeutic strategies for thoracic aortic aneurysms and dissections.

Keywords

mechanosensing; actomyosin; focal adhesion; elastic fibers; familial thoracic aortic aneurysms and dissections; Marfan syndrome

Introduction

Thoracic aortic aneurysms and dissections (TAADs) are responsible for significant morbidity and mortality in the young and old alike^{1,2}. Advances over the past 15 years in

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Disclosures:

None.

medical imaging and genetics have increased the number of diagnosed TAADs^{3,4}, which has raised the need to better understand the natural history of these potentially lethal conditions. Progressive dilatation, acute dissection, and rupture of the thoracic aorta are fundamentally biomechanical processes; in particular, dissection and rupture are extreme cases of tissue-level failures that occur when local wall stress exceeds strength. There is a need, therefore, to quantify stresses (i.e., the mechanics) within the aortic wall and to understand how the intramural cells respond to these stresses (i.e., the mechanobiology) by producing, organizing, or degrading the extracellular matrix (ECM) that endows the wall with its structural integrity.

We focus on cell-matrix interactions in TAADs and suggest that the vulnerability of the thoracic aorta to aneurysm, dissection, and ultimately rupture results, in large part, from dysfunctional mechanical homeostasis, that is, a compromised ability of intramural cells to sense their mechanical environment and to regulate the ECM to respond to that environment^{1,5}. We further suggest that actomyosin contractility, independent of tissue-level vasoactivity, is central to both sensing and regulating the mechanical state of the matrix. This interpretation leads naturally to a hypothesis that genetic mutations that affect the mechano-stimulus (e.g., stresses conveyed to cells through the ECM), mechano-sensing (e.g., via membrane receptors or actomyosin functionality), or resultant mechano-signaling (e.g., second messengers) can lead to TAADs. To support this hypothesis, we first review aortic wall composition, vessel-level mechanics and mechanoadaptations, and mechanobiological processes, and then discuss how mutant genes predisposing to TAADs can disrupt mechanical homeostasis.

Aortic Wall Composition

The thoracic aorta is a composite tube consisting of three layers: an intima comprised of endothelial cells on a basement membrane, an elastin- and smooth muscle-rich media, and a collagen- and fibroblast-rich adventitia (Figure 1). The aorta is continually subjected to complex cyclic mechanical loads from pulsatile blood pressure and flow, and, in the case of the ascending aorta, the beating heart itself. Most of the structural integrity of the aortic wall derives from myriad ECM proteins, glycoproteins, and glycosaminoglycans^{6,7}. Of the structural constituents, elastic fibers and fibrillar collagens represent ~60% of the normal wall by dry weight and dominate its elasticity (i.e., ability to distend and recoil without dissipating energy), tensile stiffness (changes in stress associated with changes in strain), and strength (maximum stress sustained without failure). Glycosaminoglycans constitute only ~3 to 5% of the normal wall, but contribute to the compressive stiffness. Most of the remaining ~35% of the wall consists of smooth muscle cells (SMCs). Vessel-level vasomotion induced by SMCs can contribute to mechanoadaptations by elastic arteries such as common carotids, and perhaps the aorta in some circumstances⁸. Nevertheless, the primary role for aortic SMCs is to govern the mechanical properties by regulating ECM in the media. Fibroblasts similarly regulate matrix in the adventitia.

Elastic fibers consist of a core of elastin (90%) surrounded by microfibrils composed primarily of fibrillins, but also fibulins and other glycoproteins. These fibers are organized within the media primarily as concentric laminae, each separated by a layer of SMCs and

associated collagens and glycosaminoglycans (Figure 1). The elastin is unique in that, under normal conditions, it is deposited, organized, and cross-linked prior to adulthood and its half-life is ~50 years in humans⁹. Consequently, elastic fibers become prestressed due to somatic growth¹⁰ and typically withstand millions of cycles of loading imposed by the heart (~34 M cycles/year in humans). The elastic fibers are linked structurally to the SMCs through oblique extensions from the elastic laminae. These extensions attach at the cell membrane to dense plaques, or focal adhesions, that connect to intracellular contractile filaments that project across the cell at the same oblique orientation as the elastic extensions (Figure 1). This configuration forms a “contractile-elastic” unit¹¹, which is a tension-bearing or -sensing structure in SMCs. The oblique orientation of this structural unit reverses direction in adjacent elastic lamellae, thus contributing to a uniform distribution of stress across the normal wall¹².

It appears that fibrillins contribute to the long term stability of elastic fibers¹³; this stability may result from connections to SMCs at dense plaques that suppress protease activity¹⁴, but the precise mechanisms remain unknown. Long-term stability also appears to result from desmosine and isodesmosine crosslinks, with fibulins promoting effective lysyl oxidase mediated cross-linking during elastogenesis¹⁵. Regardless, competent elastic fibers contribute most to aortic compliance (i.e., distensibility) and resilience (i.e., the ability to store energy during systole and to use that energy during diastole to augment blood flow). Damage to (e.g., via mechanical fatigue, that is, a loss of strength due to repeated loading) or degradation of (e.g., loss of mass via proteolysis) elastic fibers causes irreversible changes in wall structure and function.

In contrast, collagen fibers contribute primarily to overall stiffness and strength. The two primary types of collagen within the aortic wall are I (~70%) and III (~30%); the former is found primarily in the adventitia and the latter in the media where it mainly associates with elastic fibers^{6,7}. Both collagens exist as fibers, hence their structural contributions depend upon intra- and inter-molecular cross-links as well as overall fiber diameters, orientations, and interactions with other constituents. Indeed, normal fibrillogenesis requires interactions with fibronectin, other collagens (e.g., type V), and proteoglycans (e.g., biglycan), hence defects in these allied constituents can compromise collagen stiffness or strength and predispose to TAADs^{16–18}. Mutant genes encoding the procollagens for types III and I also lead to TAADs^{19–21}, particularly for type III in vascular Ehlers-Danlos syndrome. It is important that some collagen fibrils run parallel to the aforementioned contractile-elastic units in the media²².

Under normal conditions, the larger collagen fibers tend to undulate slightly and orient in a symmetric-diagonal manner with respect to the long axis of the aorta²³. This organization appears to complement the elastic fiber dependent distensibility that is favorable during systole²⁴. Loss of elastic fiber integrity can decrease the undulation of collagen fibers, which manifests at the vessel level as a loss of distensibility/extensibility (i.e., increased stiffness)²⁵. Loss of elastic fibers and increased stiffness are characteristic of aortic aging and thoracic aortic disease^{26,27}. Unlike elastic fibers, collagen fibers in the aorta appear to have a short half-life (perhaps months)²⁸ and are not affected by mechanical fatigue. Rather, appropriate turnover of collagen (deposition and degradation) is critical for aortic

homeostasis²⁹, including adaptations to altered hemodynamics or responses to disease or injury³⁰. Beyond its contribution to wall properties during normal cyclic changes in pressure, adventitial collagen plays a special role as a “protective sheath” – it bears an increasing portion of wall stress during acute increases in pressure, thus shielding medial SMCs from excessive stresses³¹. Not surprisingly, sustained increases in blood pressure can induce significant increases in adventitial collagen, which is mediated in part by increases in local angiotensin-II (Ang-II) signaling³².

Loss of elastic fiber integrity not only affects the cell biology³³, it also reduces aortic resilience, which allows the wall to dilate either uniformly, as in aging when the loss is diffuse, or locally, as in aneurysms when the loss is focal^{34,35}. In contrast, loss of collagen fiber integrity decreases strength, which can render the aortic wall vulnerable to rupture^{16,17,20,36}. Glycosaminoglycans accumulate with aging and as part of the pathology in TAADs^{26,37}, with localized pooling potentially contributing to the risk of dissection³⁸. In addition to these and other structural roles, many ECM constituents have instructional roles. Functional elastic fibers contribute to a SMC phenotype that is contractile and quiescent, thus not migratory, proliferative, or highly synthetic^{33,39}. Microfibrils sequester latent transforming growth factor-beta (TGF- β), a cytokine important to many aspects of cellular activity, including SMC differentiation and matrix turnover^{40,41}. Other growth factors and proteases are similarly sequestered by the ECM⁴². Finally, both mechanically damaged and proteolytically degraded matrix can alter the phenotype of resident cells or lead to an infiltration of inflammatory cells^{43,44}. A compromised ECM thus has mechanical and biological consequences.

In summary, medial SMCs and adventitial fibroblasts are the primary cell types responsible for establishing, maintaining, or restoring the structural integrity, and hence much of the functionality, of the normal thoracic aortic wall (Figure 1). Because the function of the aorta is largely mechanical, it is not surprising that these cells are sensitive to changes in their mechanical environment^{45,46}. Therefore, mechanics is important both for understanding the aorta as a load-bearing structure (e.g., normal distension versus dilatation, dissection, or rupture) and for identifying mechanical stimuli that drive cellular responses (e.g., homeostatic adaptations versus maladaptations or disease progression).

Wall Mechanics and Mechanoadaptations

A useful concept in biomechanics is mechanical stress, a “force intensity” having units of force per area (e.g., N/m²). Two particularly important components of aortic stress are (Figure 1): circumferential stress σ_{θ} (due to the distending blood pressure changing the circumference cyclically) and axial stress σ_z (due to forces in the direction of the long axis, including those arising from somatic growth). Other components include the radial stress σ_r (due directly to blood pressure and perivascular constraints) and wall shear stress τ_w (due to frictional interactions between the endothelium and flowing blood). Quantifying stresses as a function of position and time requires solving nonlinear differential equations, but mean values can be estimated easily in straight segments⁴⁷: $\sigma_{\theta} = Pa/h$, $\sigma_z = f/\pi h(2a + h)$, $\sigma_r \approx -P/2$, and $\tau_w = 4\mu Q/\pi a^3$, where P is the transmural pressure, a the inner radius, h the thickness of the wall, f the axial force, Q the mean volumetric blood flow, and μ the

viscosity of the blood. Of these four stresses, σ_θ and σ_z are the largest in the aorta: ~ 150 kPa under normal conditions, where $1 \text{ Pa} = 1 \text{ N/m}^2$ and $1 \text{ kPa} = 7.5 \text{ mmHg}$. Abdominal aortic aneurysms may rupture when the larger of these two stresses exceeds 450 kPa ⁴⁸; comparable values of *in vivo* failure stresses have yet to be suggested for thoracic aneurysms but are likely similar. There are, however, accumulating *in vitro* data for the human thoracic aorta on biaxial mechanical and failure properties^{49,50}. Finally, the mean radial stress is only about -6 kPa (compressive) and wall shear stress $\sim 1.5 \text{ Pa}$ (five orders of magnitude smaller than σ_θ and σ_z). Although typically neglected in analyses of wall mechanics, σ_r may be important in cases of increased glycosaminoglycans³⁸. Regardless of magnitude, all components of stress impact the mechanobiology, with endothelial cells sensitive to changes in τ_w and SMCs and fibroblasts sensitive to changes in σ_θ and σ_z .^{45,46,51}

The seminal paper by Wolinsky and Glagov¹² revealed that “the average tension per lamellar unit of [descending thoracic] aortic media was remarkably independent of species and very nearly constant” at a mean value of $T \sim 2 \text{ N/m}$. Hence, the normal number of elastic laminae is fixed for each species, including ~ 5 in murine to over 60 in human aorta. Because tension $T = Pa$ and stress $\sigma_\theta = T/h$, the $\sim 15 \mu\text{m}$ thickness of a lamellar unit⁶ yields an average circumferential stress per lamellar unit of $\sim 133 \text{ kPa}$, close to the mean value for the wall. Importantly, this finding suggests that intramural cells deposit and organize ECM during development to establish a preferred mechanical state (via morphogenesis), which they subsequently seek to maintain under normal conditions (via homeostasis).

Complementary studies further suggest that these cells seek to restore this state when blood pressure or flow increase from normal^{52,53}. Such mechanoadaptations reveal that the mature aorta retains some of its developmental ability to respond to changes in hemodynamics. Developmental adaptations include differential changes in the ascending aorta and pulmonary trunk following closure of the ductus arteriosus, whereby these two vessels adapt to their different pressures and flows⁵⁴, and differential changes in the descending thoracic aorta and infrarenal aorta following birth, when increased renal and gastrointestinal flows change the hemodynamics within the infrarenal segment⁵⁵. The most conspicuous aortic mechanoadaptation in maturity is thickening of the wall to restore intramural stresses in hypertension^{47,52}.

Maintenance of arterial caliber in response to increased blood pressure (to restore wall shear stress toward normal) tends to involve vessel-level changes in vasoactivity³⁰, which are greater in muscular arteries than elastic arteries. Although there is little information on possible basal tone in the human aorta, the murine aorta exhibits vessel-level vasoactivity (i.e., contractility) similar to that of carotids and other elastic arteries⁵⁶. Aortic contractility is diminished in the thoracic aorta of mice having mutations in genes that predispose to TAADs^{57–60}, hence contributions of shear stress mediation should be considered in these mouse models given that the ascending aorta is relatively thin, having ~ 8 medial lamellar units. The mechanoadaptive role of shear stress is less clear in human aortas because the thick media consists of 60+ layers of elastic laminae and SMCs.

The human aorta may adapt to changes in pressure and flow like other elastic arteries, yet it exhibits a distinct behavior in extreme exercise⁶¹. Marked increases in caliber manifest in the subclavian artery in the dominant arm of professional tennis players and in femoral

arteries of professional cyclists while the thoracic and abdominal aorta change modestly⁶². It may be that the extreme compliance (inverse of stiffness) of the normal aorta accommodates dramatic pressure-induced changes in flow without entrenching changes in caliber. Consistent with this idea, the aorta is responsible for ~65% of total arterial compliance⁶³; values of distensibility (e.g., $D = (d_{sys}^2 - d_{dias}^2) / d_{dias}^2 PP$, where d is diameter and PP is pulse pressure) are about 6-fold greater in the proximal aorta than in common carotids in young healthy individuals and 2-fold greater than in the descending thoracic and abdominal aorta⁶⁴. Yet, the ascending aorta may stiffen more and earlier during normal aging than either the common carotids or descending thoracic aorta^{64,65} and it exhibits earlier and more dramatic lengthening during aging⁶⁶. These changes may result from the unique biaxial loading experienced by the ascending aorta – it alone experiences cyclic circumferential stresses due to the distending pressure and cyclic axial stresses from the downward motion of the contracting heart. The ascending aorta also has the highest percentage of elastin, which, of all structural constituents in an artery, is uniquely susceptible to mechanical fatigue because of the inability to repair the elastic fibers⁹.

The unique structural and mechanical features of the ascending aorta may contribute to its vulnerability to TAADs, but this is complicated by global versus local effects. For example, thickening of the wall could restore mean values of wall stress toward normal in hypertension, but defects within individual medial lamellar units (e.g., accumulated glycosaminoglycans) could still increase stress locally and initiate a delamination³⁸. Loss of functional elastin, whether by mechanical fatigue or proteolytic degradation, would be expected to affect mechanosensing⁵ as well as contribute to arterial dilatation and lengthening²⁵, with extreme cases of the latter leading to tortuosity^{24,36}. Although decreases in the *in vivo* axial stretch of an artery tends to off-load the vessel axially and beneficially decrease both the axial and circumferential stress (due to biaxial coupling)⁶⁷, extreme cases leading to tortuosity are maladaptive. Tortuosity of other arteries, including vertebrals, may also be indicators of thoracic aortic disease risk⁶⁸. While the most dramatic effects may manifest in the thoracic aorta, other vessels can be affected⁶⁹.

In summary, aortic cells typically seek to establish, maintain, and restore a homeostatic mechanical state, which can be accomplished via matrix turnover within elastically deformed or vasoaltered states. Such a process requires intramural cells to assess the mechanical state of the ECM, then either to entrench remodeled matrix or to prestress new matrix as it is deposited. Importantly, both of these processes require actomyosin contractility separate from vessel-level vasoactivity, as discussed below. Finally, the ascending aorta appears to have a unique structure and properties consistent with its unique mechanical loading, which may render it particularly vulnerable to fatigue-induced damage of elastic fibers that, in turn, could adversely affect mechanosensing and mechanoregulation of matrix.

Mechanobiology

A seminal finding that cells respond to mechanical stimuli was the observation that SMCs change their production of ECM, including fibrillar collagens and glycosaminoglycans, in response to cyclic mechanical loading⁷⁰. Later studies showed that this SMC

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mechanobiological response occurred due to increased Ang-II signaling⁷¹, increased sensitivity to Ang-II through its primary receptor, AT₁R⁷², and increased production of a downstream effector molecule, TGF- β ⁷³. These findings were supported, respectively, by an attenuated load-induced production of matrix following treatment with an angiotensin converting enzyme inhibitor, an AT₁R antagonist, or a TGF- β neutralizing antibody. Another load-induced response by SMCs is altered production of matrix metalloproteinases (MMPs)⁷³, the potency of which can depend on mechanical loading⁷⁴. Hence, cyclic loading of SMCs affects both matrix production and degradation. Such mechanobiological effects are complicated by related effects. For example, Ang-II increases interleukin-6 and monocyte chemoattractant protein-1, which *in vivo* facilitates recruitment of monocytes/macrophages that produce cytokines and proteases that contribute to matrix turnover, particularly in adventitial fibrosis⁷⁵. Nevertheless, cyclic loading also alters SMC expression of platelet derived growth factor⁷⁶ and cationic channels involved in cell contractility⁷⁷. Figure 2 summarizes some key responses of SMCs to cyclic loading, where $\sigma(\lambda(t))$ represents the stress σ that depends constitutively on the stretch λ that varies with time t during loading. Such findings clearly link the mechanics and mechanobiology.

Similar observations hold for fibroblasts⁴⁶, which are fundamental to adventitial integrity. Fibroblasts can additionally use mechanical stresses (generated via actomyosin contractility) to activate latent TGF- β sequestered within matrix⁷⁸. Although long ignored, adventitial fibroblasts are significant contributors to aortic wall homeostasis⁷⁹, particularly in depositing, organizing, and degrading the collagen fibers that are essential for maintaining overall wall strength and preventing rupture⁸⁰. Both mechanical stress and TGF- β are required for fibroblasts to differentiate into myofibroblasts⁷⁸, which are also increasingly recognized as important in aortic mechanobiology, particularly aneurysmal enlargement in the absence of functional SMCs⁸¹.

Mechanical stimuli affect multiple signaling pathways (Figure 3), including the mitogen-activated protein kinase (MAPK) pathway downstream of Ang-II⁸² and the SMAD pathway downstream of TGF- β ⁸³. There is also cross-talk between TGF- β and MAPK signaling and between Ang-II and SMAD signaling⁸⁴. Further complicating such signaling are the following observations: influences of Ang-II on TGF- β can be mediated by epidermal growth factor and its MAPK signaling⁸⁵; elevated mechanical stress can increase local Ang-II⁷⁰ and activate latent TGF- β ⁷⁸, both of which affect MMP promoter activity⁸⁶; Ang-II can also activate latent MMP-2 while MMP-2 can activate latent TGF- β ^{87,88}; and SMCs exposed to Ang-II increase their expression of TGF- β ⁸⁹. Therefore, ECM, integrins, and growth factor/cytokine signaling are highly interconnected and responsive to mechanical loading⁹⁰.

Notwithstanding the already complex local wall mechanics and cellular mechanosensing and mechanoregulation of ECM in thoracic aortic disease, aging and hypertension are the major risk factors for TAADs^{3,4,27}. Interestingly, Ang-II and TGF- β are also important in aging²⁶ and hypertension⁹¹ wherein a switching of SMC phenotype toward synthetic⁹² can increase the production of matrix that thickens and stiffens the wall. While, thickening of the wall can favorably decrease mean wall stress locally^{30,52}, a global increase in structural stiffness can lead to increased pulse wave velocities and central pulse pressures^{91,93} that adversely

increase proximal aortic loading. Because different vascular disorders manifest in different regions (e.g., Marfan syndrome in the aortic root and vascular Ehlers-Danlos more diffusely), altered hemodynamics due to aortic stiffening need not increase stress on vulnerable regions uniformly and attempts to reduce central pressures could have differential effects. Finally, changes in stress-mediated matrix production that thickens the wall can also potentially diminish structural integrity locally (e.g., via accumulated glycosaminoglycans)^{37,38}. In summary, given the effects of stress on Ang-II and TGF- β production^{71–73}, the complex interactions amongst many related signaling pathways^{82–89}, and the differential effects of these biomolecules on different cell types^{75,78}, it should not be surprising that the roles of Ang-II and TGF- β in TAADs remain controversial^{41,94} – their roles in the basic mechanobiology and mechanics are similarly complex.

Mechanical Homeostasis Across Scales

Experiments focusing on responses of tissue equivalents, isolated cells, and sub-cellular structures to mechanical stimuli suggest that homeostatic targets also exist at these levels of organization^{95,96}. For example, fibroblasts seeded within initially stress-free but mechanically constrained collagen gels generate an endogenous stress of ~3.2 kPa that reaches steady-state within hours⁹⁷. The term “tensional homeostasis” was coined to describe this process⁹⁸. Indeed, if this stress is manually increased or decreased relative to the endogenous level, the cells quickly begin to return the stress toward the level they established originally. Not surprisingly, these cell-mediated processes depend on actomyosin contractility and appropriate integrins attaching to the ECM^{78,90} and are regulated through RhoA/RhoKinase and MAPK signaling pathways, amongst others⁹⁹. Recalling that smooth muscle contractility can contribute to vessel-level adaptations to altered hemodynamics³⁰, we see similar signaling (RhoA/RhoKinase, calcium/calmodulin, and MAPK) involved in both low (~3–5 kPa)^{97,98} and high (~100 kPa)^{100,101} contractile stress mediated ECM remodeling.

Similar to findings at the tissue level, fibroblasts decrease or increase their stiffness (which reflects changing cytoskeletal stress because of the nonlinear stress-strain behavior) in response to increases or decreases, respectively, in cell stretch¹⁰². Such findings suggest that fibroblasts also seek a tensional homeostasis, a concept that was confirmed for equibiaxially stretched SMCs¹⁰³, suggesting that a rapid disassembly/reassembly of cytoskeletal proteins (i.e., dynamic equilibrium)¹⁰⁴ allows cells to return intracellular stiffness, and hence stress, toward a preferred value following perturbations from endogenous levels. These findings are consistent with reports that individual cells generate stresses up to 5 kPa within initially unloaded 3-D hydrogels, with stress localized at focal adhesions¹⁰⁵. Such observations suggest that cells not only modify the matrix to establish a preferred micro-environment in which to reside, but also maintain their own stress at a target level (recall Newton’s third law of motion, for every action there is an equal and opposite reaction).

Nearly linear relationships have been observed between cell-induced forces at focal adhesions and focal adhesion area in fibroblasts and SMCs^{106–108}, which suggests a target value of stress around 3–6 nN/ μm^2 (i.e., 3–6 kPa). Slightly higher values (~10 to 12 kPa) were found for myofibroblasts that exhibit supermature focal adhesions and increased

actomyosin contractility relative to synthetic SMCs and fibroblasts¹⁰⁸. Nevertheless, these findings collectively suggest the existence of target values of stress. Of course, stresses at focal adhesions relate directly to stresses in the cytoskeleton, including prestresses. Indeed, it was appropriately noted that RhoA-dependent “pre-existing cytoskeletal tension affects the actomyosin apparatus, which in turn coordinates the ability of the cell to adapt to the externally applied stress”.¹⁰⁹

In summary, whether at organ (vessel mechanoadaptations), tissue (collagen gels and hydrogels), cellular (isolated non-confluent and embedded within matrix), or sub-cellular (focal adhesions and cytoskeleton) scales, a mechanical homeostasis appears to exist whereby intramural cells try to establish, maintain, or restore a preferred mechanical state^{95,96}. Whether this state is defined locally by stress, strain, stiffness, or another metric is not clear, but stress correlates well with many observations⁴⁷ (stress and strain are related via constitutive relations, thus, one can be written in terms of the other; stress and stiffness are related linearly for materials such as the aorta that exhibit an exponential stress-strain relation). Interestingly, studies at tissue⁹⁷, cell¹⁰⁵, and sub-cellular¹⁰⁶ levels all reveal a preferred level of stress ~5 kPa.

These findings, coupled with the existence of target stresses within the aortic wall of ~150 kPa, suggest that two distinct classes of SMCs (or their behaviors) contribute to arterial mechanics. First, fully contractile SMCs that generate stresses of ~100 kPa can contribute directly to overall load bearing⁴⁷, including control of caliber (recall that, at least in the mouse, tissue-level aortic contractility is similar to that in other elastic arteries^{56,58}). Second, synthetic SMCs, fibroblasts, and myofibroblasts that appear to seek a target stress of ~5 to 10 kPa cannot contribute directly to overall load bearing, but instead contribute indirectly by regulating the load-bearing ECM. That is, synthetic cells contribute to overall structural integrity by first sensing (literally probing and assessing) their mechanical environment and then producing, actively organizing, or removing ECM to support hemodynamic loads. A corollary, therefore, is that synthetic cells appear to be “stress shielded” by the ECM⁷⁸ while yet being able to sense and thereby respond to these loads. Stress shielding is consistent with the dynamic nature of mechanosensing, namely, turnover of focal adhesion and cytoskeletal proteins within minutes^{104,110}. That is, although true load-bearing must be sustained, mechanosensing can be intermittent, particularly given that adjustments via matrix turnover occur over periods of hours, days, or weeks.

Mechanosensing of Matrix

The ability of medial SMCs and adventitial fibroblasts/myofibroblasts to respond to changes in mechanical stimuli requires that they interact directly with the ECM. Their primary mechanosensors are heterodimeric transmembrane complexes called integrins (Figure 1), which are denoted $\alpha_x\beta_y$ and cluster to form dense plaques/focal adhesions¹¹¹. Vascular SMCs, fibroblasts, and myofibroblasts employ a repertoire of integrins^{78,112}, including $\alpha_1\beta_1$, $\alpha_2\beta_1$, $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_7\beta_1$ and $\alpha_v\beta_3$ by SMCs and $\alpha_2\beta_1$, $\alpha_5\beta_1$, $\alpha_v\beta_3$, $\alpha_v\beta_5$, and $\alpha_v\beta_8$ by (myo)fibroblasts. These integrins mediate binding to specific constituents of the matrix (e.g., $\alpha_5\beta_1$ for fibronectin and $\alpha_7\beta_1$ for laminin), with some overlap in specificity (e.g., $\alpha_5\beta_1$, $\alpha_v\beta_3$, and $\alpha_v\beta_5$ bind fibronectin). Although there is redundancy in genes encoding integrins,

deficiency in either σ_1 or β_1 leads to a vascular phenotype, including reduced mechanoadaptivity^{113,114}. Particular matrix constituents can influence cell phenotype and thus responses to chemo- or mechano-stimulation. For example, fibronectin tends to promote a synthetic smooth muscle phenotype whereas laminin tends to promote a contractile phenotype¹¹⁵. Importantly, microfibrils consisting primarily of fibrillin-1 connect aortic SMCs to the elastic laminae primarily via $\sigma_5\beta_1$ and $\sigma\nu\beta_3$ integrins¹¹⁶. That these microfibrils tend to align co-axially with actomyosin fibers within the cell^{11,22} suggests an efficient transfer of loads from cytoskeleton to integrin to matrix and vice versa. Loss of such connections would be expected to alter SMC signaling and phenotype⁵.

In summary, integrin engagement and actomyosin contractile activity are fundamental to mechanosensing and mechanoregulation of matrix^{99,110} and thus mechanical homeostasis^{78,96}. Findings at tissue, cellular, and sub-cellular levels of actomyosin-generated stresses of ~5 kPa are consistent with mechanosensing, not gross load-bearing. Although such active stresses are much lower than those involved in vasoactive changes of vessel diameter, the intracellular structures and signaling pathways are similar. Thus, pathways fundamental to SMC mechanobiological changes in general^{45,117} and mechanosensing in particular^{99,110} overlap considerably with those in vasoactivity^{100,101}. Similar considerations apply to fibroblasts and myofibroblasts^{78,81}.

Finally, mechanostimulation of cells involves receptor tyrosine kinases (e.g., EGFR or PDGFR), serine-threonine receptors (e.g., TGF- β type I and II receptors), G-protein coupled receptors (e.g., AT₁R and ET_A), and stretch-activated cationic (e.g., Ca⁺⁺) channels^{90,118,119}. These pathways may involve ligand-independent, mechanical activation of the receptors¹²⁰, load-induced secretion of ligand¹²¹, opening of channels¹²², or release of active ligand⁷⁸. For example, mechanical activation of latent TGF- β can be achieved via actomyosin activity and integrins that bind the latency complex (e.g., $\sigma\nu\beta_3$ and $\sigma\nu\beta_6$), which could impact adventitial remodeling³¹. Interestingly, cell generated stresses needed to activate TGF- β are ~5 to 9 kPa⁷⁸, similar to typical stresses at focal adhesions¹⁰⁶, in cells placed within otherwise unloaded matrix¹⁰⁵, and endogenous levels established in collagen gels by cells⁹⁷. It may be that mechanosensing and the mechanoregulation of cytokines⁷⁸, proteases and their action⁷⁴, or cross-linkers such as transglutaminases¹²³ that are stored within the ECM are highly complementary, again involving the same intracellular structures and signaling pathways. Finally, transmembrane polycystins appear to contribute to mechanosensing in vascular cells; they often co-localize with stretch-activated calcium channels and modulate their activity¹²⁴. Polycystin-1,2 play important roles in mechanosensing both individually (via filamin-A related interactions with cytoskeletal actin¹²⁵) and in cooperation with focal adhesions¹²⁶.

Mechanoregulation of Matrix

Theoretical studies of soft tissue growth and remodeling suggest that, under normal conditions, cells deposit new structural constituents within extant matrix at a homeostatic stress or stretch^{34,35}. This conjecture is appreciated easily in tissue maintenance: if stressed matrix is removed during normal turnover, the only way to maintain the same properties and geometry under with a similar load is to replace this matrix with the same constituents

having the same stresses. Such mechanoregulated deposition is supported computationally and experimentally. Computations of arterial mechanoadaptations show that ongoing replacement of stressed matrix with either unstressed or less stressed matrix causes a numerical artery to dilate under a constant pressure, which violates tissue maintenance³⁴. Experiments reveal that SMCs and fibroblasts mediate collagen assembly via integrins (e.g., $\alpha_2\beta_1$) and RhoA activity^{127,128}, which implicate an active process of organization. Indeed, embryonic fibroblasts can produce procollagen independent of actin, but they cannot organize and align collagen fibers without functional actin, thus implying an actomyosin-based mechanoregulation of the fibers via integrins^{129,130}.

Interestingly, TGF- β influences both $\alpha_2\beta_1$ integrin and SMC α -actin levels in cells that remodel collagen gels *in vitro*¹³¹, and focal adhesion kinase is increased in SMCs concomitant with the synthesis of proteins in response to exogenous Ang-II and its associated MAPK signaling¹³². Fibronectin is also necessary for proper collagen assembly^{127,128}, and fibronectin assembly is mechanoregulated via integrins ($\alpha_5\beta_1$) and actomyosin activity¹³³. Elastic fiber assembly is similarly mediated by cell generated mechanical loads¹³⁴. Clearly, matrix deposition and organization are mediated by active cell processes that involve force generation^{95,96}.

Cells also actively remodel extant matrix into mechanically preferred states⁷⁸. For example, fibroblasts build in a “residual matrix tension” (likely ~ 5 kPa⁹⁷) in mechanically constrained collagen gels that persists even if subsequent actomyosin activity is blocked¹³⁵. In other words, residual matrix tension preserves some cell-mediated changes in matrix organization without requiring continued actomyosin activity, which would be energetically favorable. Transglutaminases may play a role in such entrenchment just as in tissue-level arterial remodeling³⁰, noting that matrix bound transglutaminases can be activated by mechanical stress¹²³.

In summary, cells appear to modulate their ECM to match the forces encountered⁹⁹. Diverse findings suggest that when organizing either newly produced or extant matrix, cells seek to establish, maintain, or restore a preferred (homeostatic) mechanical state that correlates well with a target stress or stiffness. Fundamental to such mechanoregulation of matrix is the ability of the cells first to sense the mechanical state, which requires integrin expression and actomyosin activity. Amongst its many effects, TGF- β can increase the expression and organization of contractile proteins within SMCs and fibroblasts, which correlates with increased integrin clustering⁷⁸. It seems, therefore, that this cytokine known primarily for increasing the production of matrix simultaneously enables cells to actively organize and sense the deposited matrix through focal adhesions and actomyosin complexes. Establishing and maintaining matrix at a preferred state is fundamental to the overall structural integrity of tissues and the health of the cell. In extreme cases, however, loss of contact with matrix causes cells to undergo a special form of apoptosis called anoikis¹³⁶. Conversely, simply reducing the load in the arterial wall results in adaptive atrophic remodeling due to degradation of matrix and apoptosis^{137,138}. Clearly then, if dysfunctional mechanosensing leads cells to misinterpret a normal or high stress environment as a low stress environment, they may inadvertently apoptose or elicit an atrophic remodeling response, both of which could compromise overall structural integrity⁵.

Genes Predisposing to Thoracic Aortic Disease

Since the discovery in 1991 that mutations in *FBN1* predispose individuals with Marfan syndrome to TAADs¹³⁹, mutations in many other genes have similarly been identified^{1,27,41,69,140–147}. In addition, common single base pair variants (SNPs) and genomic copy number variants (CNVs) in some of these same genes increase the risk for TAADs in the general population^{60,148,149}. These genes can be categorized based on their effects on mechanosensing and mechanoregulation of matrix by medial SMCs, particularly through disruption of the contractile elastic unit⁵.

Numerous mutated genes predisposing to TAADs alter proteins in the microfibril sheath that encapsulates elastin (Figure 1). *FBN1* encodes fibrillin-1, the major protein in the microfibrils that connect elastic fibers to dense plaques in SMCs. Fibroblasts explanted from patients with Marfan syndrome consistently exhibit decreased deposition of fibrillin-1 into the ECM^{150,151}, thus suggesting that *FBN1* mutations compromise connections between SMCs and elastic fibers. Mutations in microfibril associated glycoprotein 2 (encoded by *MFAP5*), another component of microfibrils, also predispose to TAAD¹⁴⁷. Other mutant genes that lead to thoracic aortic disease affect proteins that normally contribute to the structural integrity of the wall (e.g., fibulin-4 (*FNLN4*), and collagen III (*COL3A1*))^{152,153}, which also connect structurally to the SMCs and could alter the mechanical stimuli sensed by these cells as the wall experiences either the same or increased cyclic loads.

Other genes predisposing to TAADs encode proteins that contribute to the structure or function of the SMC contractile unit, as well as cytoskeletal components linking these units to the dense plaques¹. The two major proteins in the contractile unit are the SMC-specific isoform of actin, α -actin, and SM-myosin heavy chain, which form thin and thick filaments, respectively. Mutations in the corresponding genes, *ACTA2* and *MYH11*, predispose to thoracic aortic disease^{60,69,142}. Preliminary data suggest that mutations in these genes disrupt the ability of monomers of α -actin and myosin heavy chain to form functional filaments. SMCs also have a dedicated kinase, myosin light chain kinase (*MYLK*), that phosphorylates the regulatory light chain (RLC) to initiate SMC contraction. *MYLK* loss of function, predicted to decrease activation of the RLC, also predisposes to TAADs¹⁴³. Type I cGMP-dependent protein kinase (*PRKGI*) controls RLC dephosphorylation to relax SMCs. A gain of function mutation in *PRKGI* that increases dephosphorylation of the RLC also predisposes to thoracic aortic disease¹⁴⁶. Finally, mutations in filamin-A, a cytoplasmic protein that links actin filaments and is strongly implicated in mechanotransduction, predispose to TAADs^{1,154}. All of these mutations in SMC contractile, structural, and signaling genes are predicted to decrease the cells' ability to generate the force needed to appropriately sense and regulate the mechanical state of the matrix.

Genetic alterations in mouse models have identified additional genes involved in ECM integrity or mechanosensing that predispose to TAADs. These genes encode other proteins and proteoglycans in the matrix (e.g., collagen V, biglycan, and lysyl oxidase)^{16,17,155}, transmembrane structures (e.g., polycystin-1,2)¹⁵⁶, intracellular signaling molecules (e.g., integrin linked kinase)¹⁵⁷, and calcium binding proteins¹⁵⁸. Interestingly, increased *S100A12* (a calcium binding protein) resulted in reduced vinculin, SM-myosin heavy chain,

and SM- α actin, each of which can contribute to effective sensing and regulation of ECM. Increased S100A12 also increased IL-6 production and activation of TGF- β pathways. Finally, TGF- β signaling in Loeys-Dietz syndrome has been suggested to be Ang-II dependent¹⁵⁹, and thoracic aortic disease in the *Fbln4*^{SMKO} model depends on intramural increases in Ang-II production¹⁶⁰.

Genome wide association studies in patients who do not have Marfan syndrome show that common SNPs in *FBNI* still associate with thoracic aortic disease¹⁴⁹. Whereas altered or diminished fibrillin-1 can contribute to premature mechanical fatigue or elastolysis, consistent with compromised wall properties expected during aneurysmal dilatation^{14,35,36} and altered mechanosensing⁵, fibrillin-1 plays additional roles – it sequesters latent TGF- β in the ECM. It has been proposed that mutations in *FBNI* can lead to inappropriate release of active TGF- β from the matrix and that excessive TGF- β signaling can cause the increased SMAD and MAPK signaling in aortas of Marfan mouse models^{161,162}. Aortic tissue from patients undergoing surgical repair of familial TAADs also exhibit increased TGF- β signaling based on immunostaining of phosphorylated SMAD2. It is thus surprising that loss of function mutations in genes encoding proteins critical for TGF- β signaling also predispose to TAADs, including those involving genes that encode type 1 and 2 transforming growth factor receptors (*TGFBR1* and *TGFBR2*), one of the three isoforms of TGF- β (*TGFB2*), and related signaling molecules, SMAD3 (*SMAD3*) and SMAD4 (*SMAD4*)^{140,141,144,145,163}. Thus, the paradox of mutations decreasing TGF- β signaling despite evidence of increased TGF- β in end stage aortic tissues raises questions as to the precise role(s) played by TGF- β signaling in thoracic aortic disease.

Studies in mice demonstrate that TGF- β signaling is critical for proper development of the aorta. Loss of TGF- β signaling disrupts the differentiation of neural crest-derived SMCs that populate the ascending aorta and the secondary heart field epithelial-to-mesenchymal transition involved in forming SMCs in the aortic root during development^{164,165}. The importance of TGF- β in the differentiation of aortic SMCs is illustrated by the finding that ascending aortas in *Tgfb2*^{-/-} mice are small and thin-walled¹⁶⁶, and mice with the *Tgfb2* gene conditionally deleted in vascular SMCs display arterial dilation and dissection⁸⁰. These studies showed that TGF- β gene mutations do not disrupt cardiovascular development; migration or homing of cells to the aorta during development was normal. Rather, decreased TGF- β signaling predisposes to slowly progressing, primarily adult-onset vascular diseases initially involving the aortic root and ascending aorta. TGF- β may be required for full differentiation of aortic SMCs during development, and specifically the development of a full complement of contractile-elastic units to withstand life-long mechanical loading of the aorta. Supporting this hypothesis, SMCs explanted from patients with *TGFBR2* mutations show reduced expression of proteins in the SMC contractile unit when compared with control SMCs, and they differentiate to a lesser degree than control SMCs in response to TGF- β ¹⁷⁶. Thus, failure to build the proper number of SMC contractile-elastic units during development due to defects in TGF- β pathway genes may lead to altered sensing of stress by the aortic SMCs and secondary compensatory increases in Ang II and TGF- β signaling.

A Unifying Hypothesis

An emerging concept is that altered cell-matrix connections, especially via the contractile-elastic unit, play important roles in TAADs^{1,14,27,58,161,167–169}. Given that such connections are fundamental determinants of cell phenotype¹¹² and cell survival¹³⁶, this hypothesis is intuitive. Based on our review of the mechanics and mechanobiology, both computational and experimental, we submit further that many of the identified genetic mutations in TAADs implicate two specific, complementary aspects of cell-matrix interactions that directly impact the structural integrity of the aortic wall. Functional connections must exist between SMCs and surrounding ECM to enable these cells (i) to actively assess their mechanical environment and express appropriate mechanosensitive genes, and (ii) to actively remodel extant matrix or prestress newly produced matrix as it is incorporated within the wall. Both of these cell-mediated functions are necessary for establishing, maintaining, or restoring structural integrity in response to altered biomechanical loads. Moreover, cell-level actomyosin contractile activity is fundamental to such mechanosensing and mechanoregulating of the matrix, as suggested by the convergence of TAAD-related signaling pathways with mechanisms of cell sensing and regulation of matrix (Figure 3). Consequently, if any one of these three essential links – matrix stiffness, transmembrane structures, or cytoskeletal structures, including the actomyosin apparatus and proper signaling to express and assemble these links – is defective (Figure 4), mechanosensing and mechanoregulation will be compromised. That is, mechanical homeostasis will be dysfunctional and the aorta will become vulnerable to structural failure.

Given the apparent importance of TGF- β and possibly Ang-II in TAADs^{1,27,41,161,170}, their mechanobiological roles must be considered. To this end, *in vitro* and *in vivo* studies reveal direct relationships between mechanical stimuli on SMCs and both Ang-II and TGF- β activity^{71–73,117}. These results are consistent with the influence these biomolecules have on matrix production/degradation^{78,87,89} and the actomyosin-integrin structures^{82,83} that are needed to organize a matrix capable of supporting hemodynamically induced loads. Indeed, Ang-II and TGF- β play similarly important roles in hypertension and aging wherein the wall can thicken to counteract increased pressure-induced wall stress^{26,32,91,93}, though not all matrix production need be structurally protective^{37,38}. Interestingly, a transcriptional analysis of vascular aging identified altered cell-matrix interactions as particularly important¹⁷¹. Mechanical stress and TGF- β signaling are also complementary partners in the differentiation of fibroblasts to myofibroblasts, which enables increased synthesis and organization of matrix^{78,81}. These processes may play important roles in adventitial remodeling in severe cases of increased mechanical stress^{32,75,80}, which, when the media degenerates in TAADs, is engaged as a protective sheath³⁵.

Closure

The normal thoracic aorta can maintain its geometry, structure, and function over decades and adapt well to modest sustained changes in hemodynamics. Such maintenance and adaptivity are achieved, in large part, by intramural cells properly sensing their chemo-mechanical environment and actively regulating the ECM to ensure appropriate compliance and structural integrity. However, aging results in diffusely diminished smooth muscle

functionality, lost elastic fiber integrity, remodeled collagen, increased glycosaminoglycans, and increased intramural Ang-II and TGF- β ^{26,171}. These changes can manifest clinically as decreased arterial distensibility, increased pulse wave velocity, and increased central systolic and pulse pressure, all of which feedback to increase the hemodynamic loads⁹³. Many of these same changes are found at tissue, cellular, and molecular levels in some individuals having TAADs^{142,172}. In some cases, therefore, TAADs may represent an accelerated, exaggerated “localized aging” of the aorta. In other cases, mutations (e.g., in *TGFBR1/2*) likely compromise the developing matrix and differentiation of SMCs, which affects subsequent function and structural integrity⁸⁰, including arterial tortuosity at young ages². Thus, ineffective cell-matrix interactions likely render the wall vulnerable to increases in hemodynamic loading, even during development.

The aortic wall will fail as a structure, namely dissect or rupture, if and only if local mechanical stresses exceed mechanical strength. There is a pressing need, therefore, to focus on local, not global, mechanics¹⁷³. In contrast, mechanobiological responses by aortic cells need not depend on the actual mechanical environment – responses depend on what the cell can perceive (i.e., sense) and achieve (e.g., genes that can be expressed). Based on the diverse findings collected together herein, we submit that TAADs arise, in large part, due to an inappropriate mechanosensing and mechanoregulation of ECM by medial SMCs that renders the aortic wall vulnerable to dilatation and dissection. An associated or subsequent inability of adventitial fibroblasts to maintain or restore a sufficiently strong adventitia can further lead to rupture.

Future research should thus focus on local, not global, mechanics; mechanisms by which cells sense changes in local mechanical stimuli; and associated signaling and gene expression pathways that govern the structural integrity of the aortic wall. Some guidance forward is found in the recent elucidation of abnormal mechanosignaling in the heart in Marfan syndrome¹⁷⁴. Elucidation of these pathways may identify new therapeutic targets to decrease destructive (low-stress) or enhance constructive (high-stress) mechano-sensitive remodeling of matrix. On this foundation, translational efforts to prevent TAAD may be successful in preventing aneurysms and dissections of the thoracic aorta.

Acknowledgments

Sources of funding

This work was supported by grants from the National Marfan Foundation (JDH and GT), the John Ritter Foundation (DMM) and the NIH: R01 HL062594 and P50 HL083794 (DMM), R01 HL075092 (MAS), and U01 HL116323 (JDH and GT).

Nonstandard Abbreviations and Acronyms

Ang-II	angiotensin-II
AT₁R	angiotensin-II type I receptor
CNV	copy number variant
ECM	extracellular matrix

MMP	matrix metalloproteinase
RLC	regulatory light chain
TAAD	thoracic aortic aneurysms and aortic dissections
TGF-β	transforming growth factor - β
SMC	smooth muscle cell
SNP	single nucleotide polymorphism;

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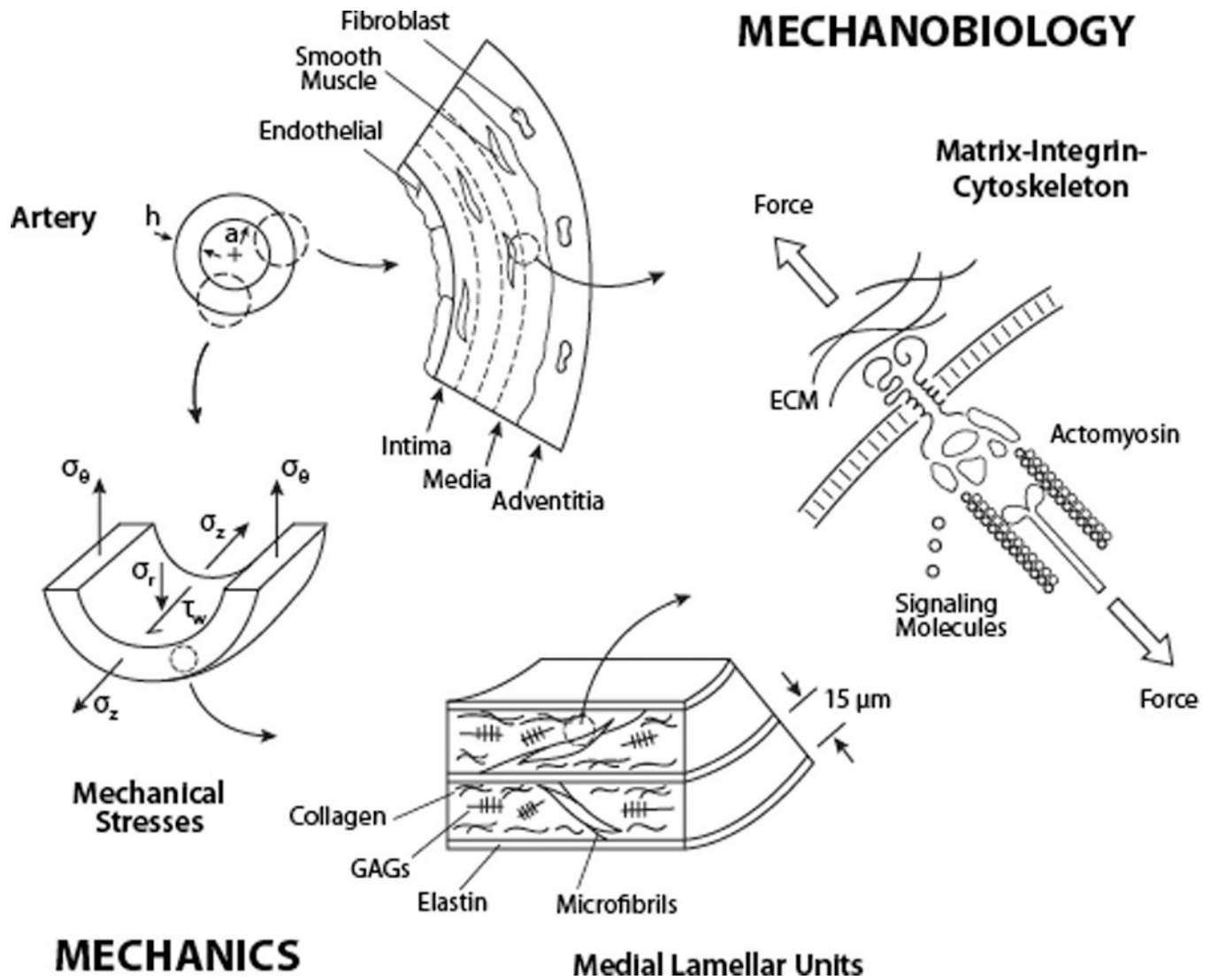


Figure 1.

TAADs manifest clinically at the macro (tissue) level but result from mechanisms at the micro (molecular) level. Shown are aortic structure and relevant mechanical stresses (see text) that result from hemodynamic loading. The stressed wall consists of three layers (intima, media, adventitia) populated, respectively, by three cell types (endothelial, smooth muscle, fibroblasts). Medial lamellar units consist of many concentric delimiting elastic laminae (elastin plus microfibrils), with associated smooth muscle cells, collagens, and glycosaminoglycans. A unique contractile-elastic unit consists of co-linear extracellular microfibrils connecting through heterodimeric transmembrane complexes (integrins) with intracellular actomyosin filaments to create load-bearing or sensing functions. Linker proteins within the focal adhesion have structural or signaling roles.

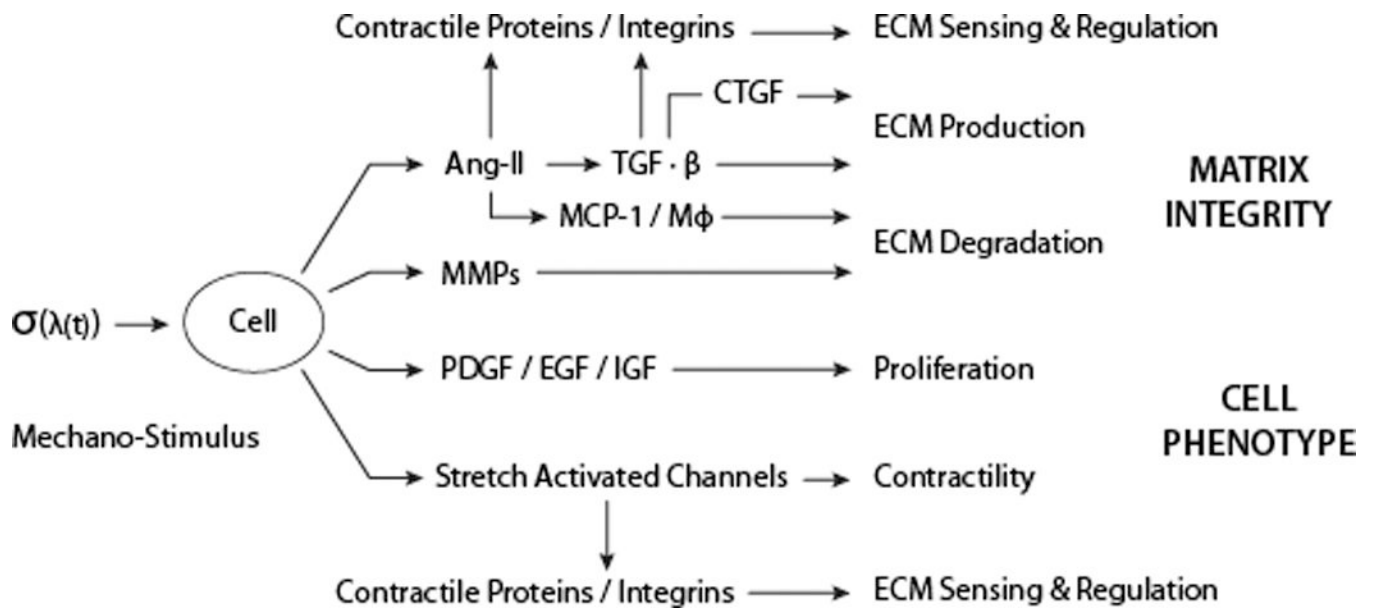


Figure 2.

Some of the many smooth muscle cell responses to an applied stress (σ) or stretch (λ). Angiotensin II (Ang-II) is a potent vasoconstrictor that also regulates both the production of intracellular contractile and extracellular matrix (ECM) proteins (partly through transforming growth factor-beta, TGF- β , and connective tissue growth factor, CTGF) and the removal of ECM (partly through monocyte chemoattractant protein-1, MCP-1, and thus monocytes/macrophages, M Φ). Matrix metalloproteinases (MMPs) contribute further to matrix removal whereas platelet derived growth factor, PDGF, epidermal growth factor, EGF, and insulin-like growth factor, IGF, contribute to cell proliferation. Finally, stress/stretch activated ionic channels also play important roles in contraction.

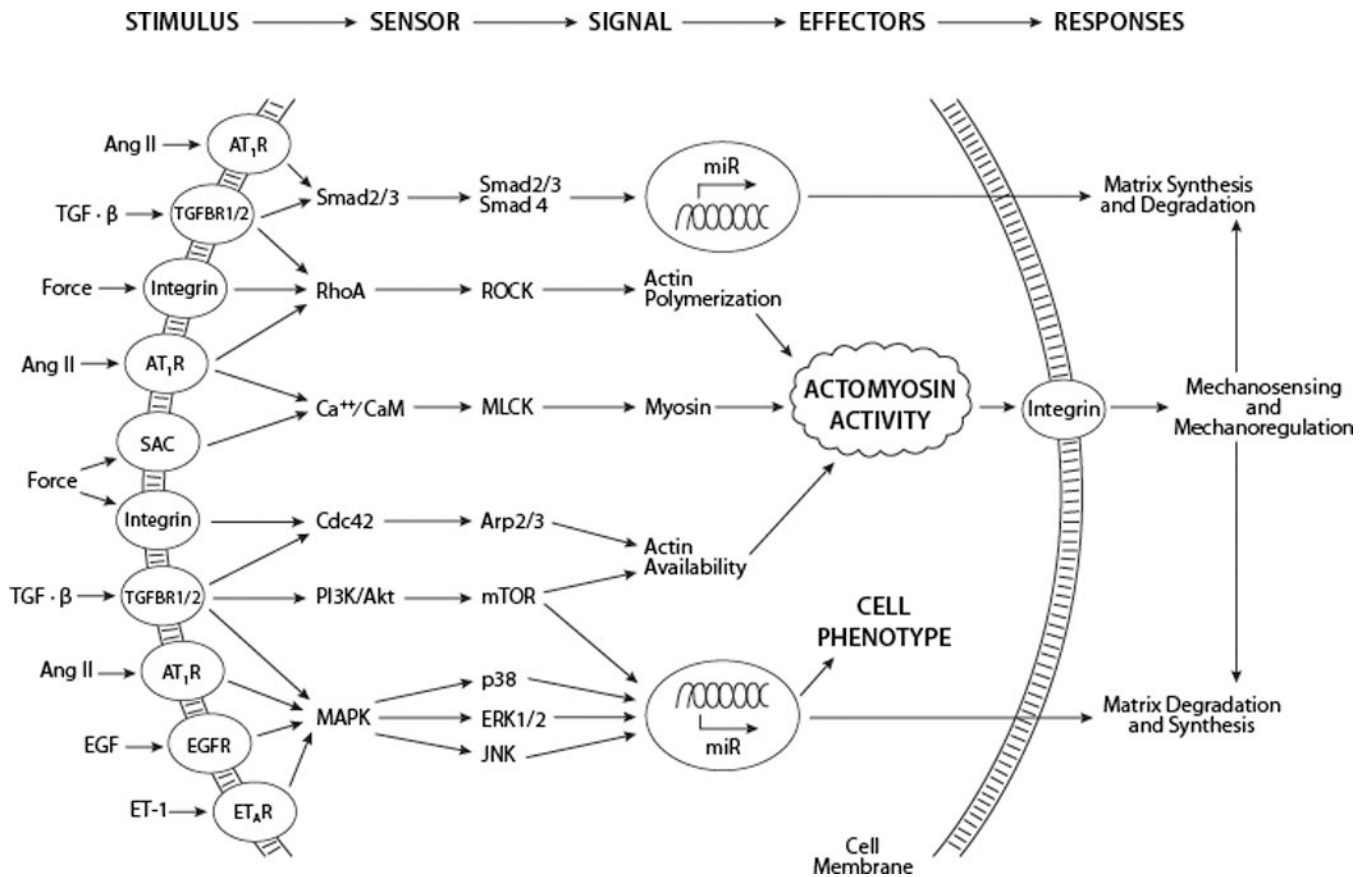


Figure 3.

Some of the many membrane receptors and associated signaling pathways that are activated by chemo-mechanical stimuli. In many cases, these diverse pathways alter the actomyosin activity that is fundamental to cellular sensing and regulating of the extracellular matrix, which in turn is fundamental to defining aortic compliance and structural integrity. Other pathways, including NF- κ B, are similarly important, particularly because of associated inflammation¹⁷⁵.

ALTERED MECHANO-SENSING and MECHANO-REGULATION OF MATRIX

Mutant Genes Predisposing to TAADs

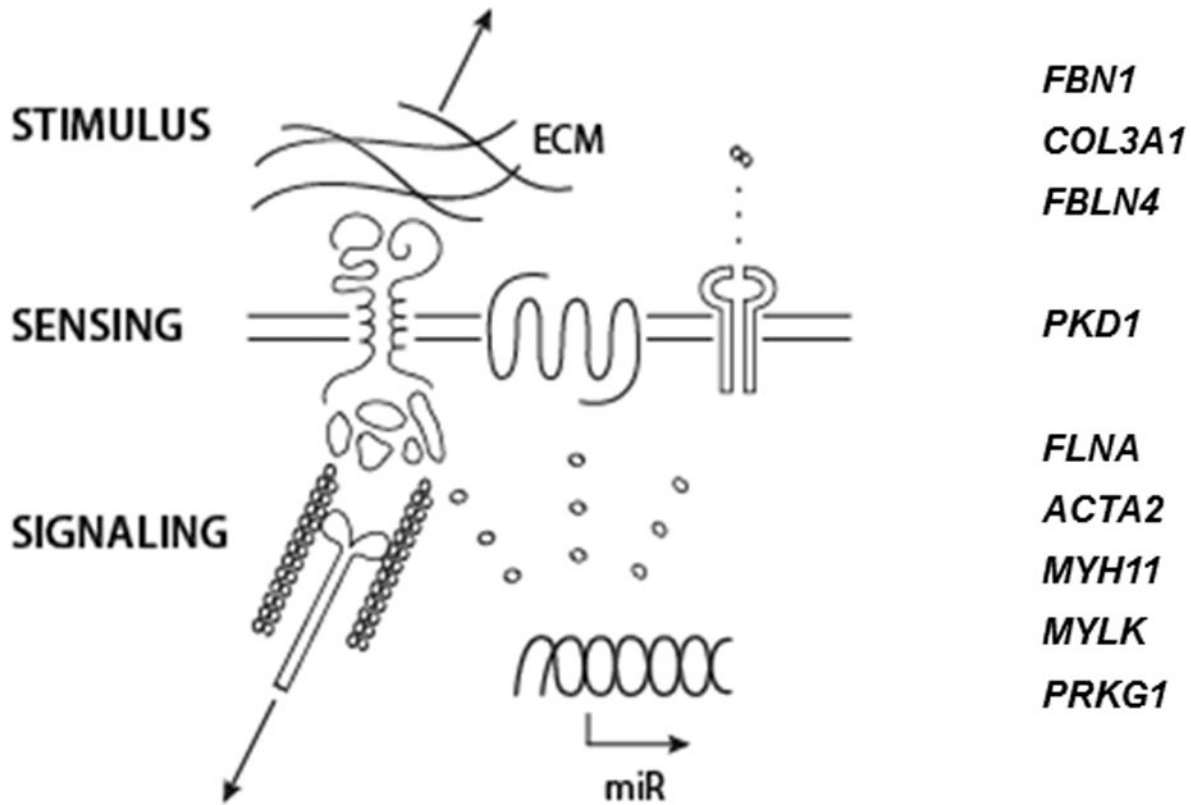


Figure 4.

Confluence of some of the gene mutations that predispose to thoracic aortic aneurysms and dissections and affect the: mechano-stimulus (e.g., transfer of stress from the matrix to the cell), the mechano-sensing of this stimulus, or the associated mechano-sensitive signaling pathways. See text for information on the affected genes and related references.