Invited Review

Extracellular matrix synthesis in vascular disease: hypertension, and atherosclerosis

Markella Ponticos[a], Barbara D. Smith[b, ✉]

[a]*Centre for Rheumatology & Connective Tissue Diseases, Division of Medicine-Inflammation,*
Royal Free & University College Medical School, University College London, London NW3 2PF, UK;
[b]*Department of Biochemistry, Boston University School of Medicine, Boston, MA 02118, USA.*

Abstract

Extracellular matrix (ECM) within the vascular network provides both a structural and regulatory role. The ECM is a dynamic composite of multiple proteins that form structures connecting cells within the network. Blood vessels are distended by blood pressure and, therefore, require ECM components with elasticity yet with enough tensile strength to resist rupture. The ECM is involved in conducting mechanical signals to cells. Most importantly, ECM regulates cellular function through chemical signaling by controlling activation and bioavailability of the growth factors. Cells respond to ECM by remodeling their microenvironment which becomes dysregulated in vascular diseases such hypertension, restenosis and atherosclerosis. This review examines the cellular and ECM components of vessels, with specific emphasis on the regulation of collagen type I and implications in vascular disease.

Keywords: extracellular matrix, vascular disease

INTRODUCTION

The vertebrate vascular network is a closed system which functions to circulate nutrients, oxygen, and immune cells to all the tissues in the body and to transport metabolites for detoxification in the liver and excretion in the kidneys. There is a hierarchical network of three main types of blood vessels, arteries, veins and capillaries characterized by location, size, function, and structural composition. Vessels are composed of an endothelial cell layer that is in direct contact with blood flow through the lumen. One of the main roles of this layer of cells is to function as a selective barrier between the blood stream and tissues. This single layer of cells is surrounded by an extracellular matrix (ECM), a basement membrane, followed by layer(s) of mesenchymal cells either smooth muscle cells (SMC) or pericytes. The mesenchymal cells are embedded in a complex ECM whose composition varies depending on the size of the vessel. This ECM contains elastic microfibrils (elastin, fibrilins), collagens (mainly types I, III, IV, V and VI), matricellular proteins (fibronectin, tenascin, and thromospondin), growth factors and proteases sequestered in matrix, and proteoglycans. This review focuses on vascular ECM function and synthesis dur-

✉Corresponding author: Barbara D. Smith, Ph.D, Department of Biochemistry, Boston University School of Medicine, K420,70 East Concord Street, Boston, MA 02118, USA. Tel/Fax: 1-617-638-4159/1-617-638-5339. E-mail: bdsmith@bu.edu.

ing vascular disease with particular emphasis on type I collagen, the most abundant matrix protein synthesized in vascular fibrosis.

THE ORGANIZATION OF VASCULATURE NETWORK

Vascular ECM and mechanics in large arteries has been extensively reviewed by Wagenseil and Mecham[1-3]. Large muscular arteries close to the heart are often called elastic arteries that provide elastic distention and recoil[3]. These arteries are composed of three structural layers known as tunicae. The innermost layer, the tunica intima, is lined with endothelial cells that produce and attach to a basal lamina, with collagen type IV and laminin, supported by an internal elastic lamina. There are anchoring and connecting microfibrils with fibrillin, elastic fibers, and a separate collagen fiber network[4,5]. Since endothelial cells can synthesize elastin and collagens[6,7], they, most likely, contribute to the subendothelial layer and internal elastic lamina. There are sporadic SMCs present in some human tunica intima.

The middle layer, tunica media, is primarily composed of circumferentially aligned SMCs and elastin which is arranged in fenestrated sheets or lamella into a three dimensional continuous network between collagen fibers and thin layers of proteoglycan-rich ECM[3]. The number of lamellar units, defined as an elastic lamella and adjacent SMCs, is related linearly to tensional forces within the wall with the greatest number of elastic layers in the more proximal vessels that experience the highest wall tension[8]. Elimination of the SMCs from large aortas does not alter the static mechanical properties of mature aortas, suggesting that the mechanical characteristics are primarily due to the ECM that is about 50% of the large vessel weight.

The outermost layer, tunica adventitia, is a collagen-rich area outside the external elastic lamina containing a heterogeneous mixture of myofibroblast cells arranged longitudinally. The relatively high content of collagen fibrils, primarily collagen types I and III, in the adventitia helps prevent vascular rupture at high pressures. The amount of collagen determines the tensile strength of the artery.

Arterioles contain endothelial cells and SMCs with less elastin and collagen. Smaller blood vessels (microvessels or capillaries) have endothelial cells surrounded by basement membranes devoid of typical SMCs. Instead, there are other perivascular cells, called pericytes, located adjacent to the vascular endothelium. These cells are also present in continuous fenestrated arterioles, capillaries, and venules less than 30 microns in diameter[9].

CELLUALA COMPONENTS OF THE VASCULATURE

The vascular network develops through different embryonic origins[10]. Endothelial cells are heterogeneous[11] and function to regulate vascular permeability (reviewed by Goddard and Iruela-Arispe[12]). Endothelial cells produce basement membranes at their basal surface and recruit mesenchymal cells during development. Endothelial cells are largely responsible for expansion of vascular beds through angiogenic process which is greatly dependent on ECM remodeling as reviewed recently[13]. A full description of the process of angiogenesis is beyond the scope of this review. Aging impairs the structure, function, and regeneration of the endothelium[14]. Senescent endothelial cells secrete increased inflammatory molecules and matrix remodeling enzymes, MMPs[15,16].

The lineage of the SMCs by fate mapping (as reviewed by Majesky[10]) indicates that SMCs originate from several locations which may in part explain the heterogeneity in the vasculature and cells. For example, the proximal aorta originates from neural crest forming the ascending and arch of the aorta. The secondary heart field forms the cells in the base portion of the aorta and pulmonary trunk while somites form the upper dorsal aorta. Finally, the splanchnic mesoderm forms the lower dorsal aorta. In fact, there are lineage specific differences in the proliferation and transcriptional responses to growth factors such as TGF-β[17]. SMCs in mice produce ECM during embryo development through 7-14 days after birth. The synthesis of most of these proteins, including collagen and elastin, rapidly decreases over 2-3 months after birth until in adult mouse turnover of ECM is low[3,18]. SMCs within the tunica media are two general types: contractile cells that respond to agonist-induced contraction and synthetic cells that synthesize ECM[19]. SMCs in culture and during development exhibit the synthetic phenotype whereas in adult vessels they are primarily contractile. Following vascular injury, SMCs demonstrate a remarkable plasticity to switch phenotypes[20,21]. Markers of the SMC phenotype include α smooth muscle actin (SMA), transgelin or SM22α (TAGLN1), calponin (CNN1), and smooth muscle myosin heavy chain (MYH11). SMCs maintain lower amounts of these proteins when they become synthetic producing less basement membrane with more protein synthesis and lack of organized contractile apparatus within the cytoplasm. Therefore, they are difficult to distinguish between synthetic SMCs and myofibroblasts, fibroblast-like cells with smooth muscle specific proteins, such as SMA, that

synthesize type I collagen in granulation tissue and contracture of wounds[22,23]. Vascular smooth muscle cells undergo cellular senescence and secrete inflammatory cytokines (IL-1, IL-6/8, MCP-1) and remodeling proteases, (MMP-2) with a similar phenotype to senescent fibroblasts refered to as age-associated arterial secretory phenotype[14].

The adventitia layer contains a mixture of cells, predominantly fibroblast and myofibroblast cells, that produce the majority of the fibrillar collagen necessary for tensile strength of the artery. The adventitia also plays an active role in vascular remodeling following injury and stress[24-26]. Perinatal and adult vessels contain a subset of SMC progenitors with stem cell markers that remain in the vessels to respond to stress and injury[27]. These cells are capable of migration into the medial and intimal layers of the arteries as reviewed by Majesky[24,28]. The adventitia is an active compartment for inflammatory cells trafficking into and out of the vessel wall along with maintaining a microvascular network that provides for trafficking with the surrounding tissue. This layer has abundant ECM that interacts with cells inside and outside the vessel providing the conduit for these interactions. The ECM is dynamic, not static[29] and sequesters growth factors such as PDGF or VEGF[30,31] and enzymes which are activated and/or carried to cells by ECM components.

Small blood vessels and capillaries where adventitia and SMCs are absent contain pericytes[9,32]. These cells are intimately associated with endothelial cells within a surrounding basement membrane. Immunochemistry indicates they are similar to myofibroblasts containing SMC α-actin, tropomysin and desmin. Pericytes are crucial regulators of microvessel integrity that control vessel contraction and permeability[12].

VASCULAR ECM COMPONENTS AND FUNCTION

Fibrillins are a class of microfibrils found widely distributed in many organs often associated with elastic fibers in the vascular system[33-35]. Fibrillin mutations and mouse gene targeting causes Marfan´s disease where the vascular tissues have disorganized and fragmented elastic fibers causing dilated aortas that can progress to dissection and spontaneous rupture of the vessel wall[36]. Microfibrils also contain microfibril-associated proteins including latent transforming growth factor binding proteins, emmilins, microfibril-associated glycoproteins as reviewed by Kielty et al[37]. The bioassembly of these proteins as a microfibril associates with proteoglycans and latent TGFβ binding protein which sequesters inactive TGFβ and bone morphogenic proteins (BMPs) as recently reviewed by

Ramirez and Sakai[38,39]. These assemblies contribute to the extracellular regulation of TGFβ and BMPs by controlling activation and bioavailability of the growth factors. Fibrillin microfibrils are present throughout the vasculature interacting with cells through integrin (αvβ3)[40], which is important in regulating vascular SMC migration, proliferation, adhesion and survival. Microfibrils, thereby, play an important structural and instructive role for vascular cells.

Fibrilin microfibrils are closely associated with elastin, a cross-linked polymer, forming the elastic fibers and elastic lamella within large vessels[3]. Elastin is distensible with a low tensile strength which functions as an elastic reservoir and distributes stress evenly throughout the aortic wall on to collagen fibrils. Elastin haploinsufficiency is sufficient for the development of supravalvular aortic stenosis[41-43]. Loss of elastin gene in mice is lethal due to obstructive arterial disease with subendothelial cell proliferation of SMCs in the media. With half the normal elastin, mice adapt forming additional SMC layers, but the vessel wall is thinner and lumen is smaller. The mechanical stress due to the lowered elasticity during development stimulates SMC proliferation to form working vessels with a similar vessel wall to the luminal diameter in wild type. Mice with no elastin die within a few days of birth due to SMC overproliferation that eventually occludes the vessel lumen[44]. The mechanical stress/strain relationships and mathematical models in growth and remodeling of large vessels has been recently reviewed[1,3].

Proteoglycans found in a vessel wall are large aggregates interacting with hyaluronic acid, small leucine-rich proteoglycans and cell surface-associated proteoglycans. The large aggregates form an extensive, interconnected polymeric network in the extracellular space primarily in the basement membrane. The small leucine-rich proteoglycans bind other ECM molecules such as fibrillin, collagen or fibronectin. These have been shown to sequester growth factors or regulate collagen fiber size[45] and are located in all layers. In addition, there are important cell associated heparan sulfate proteoglycans, and syndecans, which interact with growth factors or chemokines to signal through cytoplasmic domain and regulate vascular cell migration, angiogenesis and function during remodeling. Null mutations of syndecans in mice produce vascular irregularities[46]. Multiple additional ECM proteins that modulate cell-matrix interactions and cell function, often referred to as matricellular proteins, are also present within the vascular extracellular network with a regulatory function. These include fibronectin[47] thrombospondins[48-50], SPARC[51], tenacin-C[52], ACLP[53,54,

and CCN2[55]. In addition, proteases, such as metallo-proteinases (MMPs), are sequestered in a latent form, inactivated by tissue inhibitors of MMPs (TIMPs) in ECM until needed for remodeling[56]. Additional proteases are present that convert ECM molecules to more active molecules such as BMP1 and ADAMTS-2 that convert procollagen to collagen in the ECM. Additional enzymes present in the ECM include enzymes that modify ECM molecules such as lysyl oxidase necessary for collagen and elastin crosslinks. All these enzymes are necessary for remodeling matrix.

The collagens are a large family of ECM proteins with at least 27 collagen types composed of about 42 distinct polypeptide chains[57]. Expression array data identifies 17 different collagen types in the mouse aorta. Collagens I, III, IV, V and VI have the highest expression[58]. Collagen type IV is located primarily in basement membranes at the basal layer of the endothelial cells and surrounding SMCs. Collagen VI forms a unique fibrillar structure that associates with fibrillin microfibils to connect elastic lamellae to the basement membrane of SMCs or to other structures[47]. Large collagen fibrils contain multiple fibrillar collagens type I, III, and V within heterotypic fibrils. The actual composition of the collagen fibrils differs by specific regions of the vascular tree possibly based on derivation of cells forming the vessels. Collagen type V initiates collagen fibril formation[59]. Therefore, mice deficient in type V collagen die due to lack of collagen fibril formation. Heterozygous mice for loss of collagen type V function have vessels with decreased stiffness[60]. Mice deficient in collagen type III have a vascular defect with fragile blood vessels and a propensity towards rupture in large vessels[36]. Collagen type III co-localizes with collagen type I fibrils primarily in the adventitia. Collagen type I mutations have a wide variety of phenotypes in skin, tendons and bone, with vascular implications including aortic dilation dissection and rupture[36]. The turnover of collagen content is slow (~10 years) and with time collagen fibrils become increasingly crosslinked with collagen crosslinks such as pyridinoline and the addition of sugars referred to as advanced gycation end products (AGE) which is thought to increase vascular stiffness[61]. These studies emphasize the importance of collagen fibrils for normal mechanical structural integrity. Most importantly, many studies establish a role for collagens in vascular cell signaling of proliferation, migration, and adhesion through the β-integrin family and discoidin-domain receptor family. The ECM can be thought of as a dynamic complex of molecules that not only determine the mechanical properties of the vasculature but also regulate vascular cell activity through direct interaction with cells or by sequestering, activating or delivering growth factors to cells.

MECHANICS AND MECHANOTRANSDUCTION IN BLOOD VESSELS

Blood vessels are exposed to radial, axial, and circumferential strain generated by pulse pressure[62]. This stretching pressure is felt by the circumferentially arranged SMCs and adventitial cells through their interaction with ECM. There are local deformations in individual ECM fibers attached to membranes through transmembrane proteins such as integrins. The deformation causes conformational changes in ECM proteins and the integrins, triggering focal adhesion complex formation. Integrins are directly connected through the focal adhesions to cytoskeletal proteins that link all the way to the nucleus resulting in changes in nuclear shape and altered gene expression (reviewed in Gieni and Hendzel[63]). To explain continuity and transmission of mechanical signals, Ingber proposed a tensegrity or tensional integrity model of cellular architecture based on a synergy between balanced continuous tension and non-continuous compression components[64-66]. Vascular cells have a cytoskeleton of actomyosin contractile filaments that cause tension with cross-linked bundles of microfilament and microtubules. Vascular SMCs remain as contractile cells when responding to normal pulsation and shear forces.

The actin cytoskeleton is likely the transducer of signals to the nucleus. Whether through stretch[62] or tensile strength[67] applied to vascular SMCs, Rho activation induces cytoskeletal tension and cell stiffness with increased stress fibers, myosin phosphorylation and integrin activation. Two mechanosensitive systems of tension have been described. The first is Yorkie-homologues YAP (Yes-associated protein) and TAZ (transcriptional coactivator with PDZ-binding motif, also known as WWTR1) as nuclear relays of mechanical signals exerted by ECM rigidity and cell shape[68]. The second, demonstrated first in SMC cells, is the serum response (SRF) myocardin pathway[69-71]. In fact, the primary SMC contractile genes involved in SMC differentiation are regulated by stretch induced RhoA/ROCK pathway. The promoters of multiple SMC marker genes such as SMA and SM22 all have a pair of CArG-box motifs, $(CC(A/T)_6GG)$, which are necessary for expression[70]. Serum response factor (SRF) binds to CArG-boxes and recruits myocardin family members, which activate multiple SMC specific genes[72]. Several pathways repress transcription of SMA by altering SRF binding. Most notable, there is a GC rich region that recruits Kruppel-like factor (KLF4) or SP1 transcription factors with a histone deacetylase

(HDAC2) that deacetylates chromatin repressing transcription when treated with PDGF or oxidized lipid. In addition, SRF can form a ternary complex with Elk rather than myocardin which activates proliferation through activation of early response genes such as c-Fos[69]. Myocardin is the first identified member of a family of co-activators responsible for tissue specific regulation of cytoskeleton molecules (as reviewed by several investigators[73-75]). There are 4 cardiac and smooth muscle cell isoforms of myocardin[76]. The isoforms interact with different NK homeobox genes to regulate specific cardiac or smooth muscle cell actins[77]. The SMC specific myocardin isoform is critical and sufficient for a SMC contractile phenotype[78,79] through interactions with SRF.

The two other myocardin family members, called myocardin related transcription factors (MRTF)-A (MKL1/Bsac/Mal) and MRTF-B (MKL2), interact with globular actin in the cytoplasm. Translocation to the nucleus occurs following Rho family activation and polymerization of actin cytoskeleton into stress fibers linking actin dynamics to transcription[73]. These related factors are the proteins that respond to stretching or force[80]. MRTF-B is required for vascular development and differentiation of smooth muscle cell[81]. Loss of function of MRTF-A is not lethal and knockout mice have no myoepithelial cells in mammary glands so the homozygous mice cannot nurse[82,83]. Most importantly, there is a reciprocal relationship of MRTF-A with myocardin and MRTF-B during the phenotypic switch that occurs in pathological vascular diseases[84].

The SRF/myocardin pathway also regulates two conserved vascular microRNA genes miR-143/145 and miR-1 that regulate contractility and are down regulated during neointimal expansion and phenotypic switching[85-88]. SRF regulates multiple microRNAs including miR-29[89]. ECM molecules as a group are regulated by the miR-29 family[90]. Multiple ECM proteins are direct targets of miR-29 so that a single microRNA has a broad range of activities on functional related genes[91]. Aberrant regulation of miR-29 contributes to disease processes. Down-regulation of miR-29 in the heart after myocardial infarction cause fibrosis. MiR-29 is down-regulated in multiple diseases leading to fibrosis such as scleroderma, pulmonary fibrosis and liver fibrosis[92-97]. In addition to ECM, miR-29 targets proteins involved with immune response, proliferation apoptosis and differentiation. There is a recent review of the miR-29 family[91].

In vascular remodeling in atherosclerosis, restenosis and hypertension, the major fibrotic response of the vasculature is an increase in myofibroblast differentiation in the adventitia. MRTF-A is critical to TGF-β and matrix stiffness-induced myofibroblast differentiation[98,99]. Myofibroblasts are capable of contracting granulation tissue through Rho/Rac signaling[100]. This contraction is responsible for conversion of latent TGF-β in the ECM to active growth factor by myofibroblasts through interaction at the cell surface through integrins[101]. A review of recent developments covers the important signaling and epigenetics in this cell type in several tissues[102]. Multiple ECM molecules important in vascular biology are synthesized by these myofibroblasts under tension as reviewed recently[103].

VASCULAR FIBROSIS

Vascular fibrosis involves accumulation of ECM, particularly collagen in the vasculature due to remodeling in aging hypertension[104], restenosis[105], and atherosclerosis[106]. Following vascular injury due to increased stress or age there is an influx of inflammatory cells which can occur in the adventitia as well as through the endothelium[104]. Several risk factors for vascular fibrosis include Renin-angiotensin-aldosterone system, hyperhomosysteinemia, dyslipidemia, hyperglycemia which stimulate TGF-β and CTGF expression[55,104] which are important in development of fibrosis.

Age associated changes in cells and ECM occur in the vasculature due to constant remodeling throughout a lifetime[14]. The endothelium becomes dysfunctional from constant stress. Vascular smooth muscle cells proliferate and become more secretory producing more collagen. Both cell types become senescent promoting inflammatory cytokines, growth factors, and ECM remodeling factors referred to as senescence secretory phenotype (SASP)[14,107,108]. Senescent vascular smooth muscle cells overexpress collagen and genes involved with bone calcification (RUNX, BMP2 and alkaline phosphatase)[109]. Lamin A, a nuclear envelope protein, has been implicated in several disorders including forms of progeria or premature aging. The precursor form of lamin A, prolamin A, accelerates vascular calcification as part of the vascular smooth muscle cell senescence[110]. Lamin A/C regulates the SRF/MRTF-A mechanotransduction pathway[111] and inactivation of SRF in mice leads to decreased vascular tone and arterial stiffness. Vascular smooth muscle cells increase MRTF-A as they become synthetic and MRTF-A activates collagen gene expression[112] to accelerate remodeling. As remodeling implies, ECM is degraded and reformed during remodeling. Degradation of matrix involves primarily the matrix metalloproteinase (MMP) enzymes[113]. As a result, elastin becomes fragmented within larger arteries. Collagen becomes more crosslinked with increased glycation

that is associated with increased cellular dysfunction through receptor (RAGE) mediated processes[114]. The arterial stiffening that occurs with age is partially due to these ECM changes[114] and cellular tone.

Hypertension is often an age-related process which, in the elderly, is associated with arterial stiffness characterized by fissuring and fracturing of the elastin protein, collagen proliferation, and calcium deposition. The tension of the arterial wall alters as the vessels dilate leading to increased pulsatile stresses and further degeneration of the extracellular matrix artery. In contrast, hypertension in young subjects may be caused by increased resistance in small vessels causing an "upstream" increase in pressure at the level of the large elastic arteries which stretch and become stiffer. This type of hypertension progressively leads to vascular remodeling, hypertrophy, and hyperplasia. As yet the etiology and the pathways that lead to the development of hypertension are unclear and the relationship between vascular remodeling, arterial stiffness and hypertension is unknown. Therefore, one can only speculate whether arterial stiffness is the result of hypertension or is its cause with evidence available to support both arguments[115,116].

Pulmonary arterial hypertension (PAH) is a general term for abnormally elevated pressure in the arteries of the lungs and is simply defined as an elevation in mean pulmonary arterial pressure (PAP) above 25 mmHg at rest or 30 mmHg with exercise which creates added strain on the right side of the heart. Pulmonary hypertension (PH) is a hallmark of PAH, but PH includes all cases of increased pulmonary arterial pressure (PAP), regardless of its cause. The etiology and pathobiology of this rapidly progressive and ultimately fatal disease is complex and poorly understood with current treatments relatively ineffective[117]. PAH includes a heterogeneous group of conditions which despite the diversity is defined by similarities in pathophysiological, histological and prognostic features[118,119]. PAH is characterised by severe arteriopathy, including increased thickness of the intima, media and adventitia of peripheral arteries, muscularization of the precapillary arterioles and capillaries which result in narrowing and/or occluding pulmonary arteries and arterioles, the obstruction of blood flow and ultimately loss of distal vasculature. The pathology contributes to vascular lesions (e.g. plexiform lesion and neointimal proliferation), which also obstruct the pulmonary arteries and arterioles. The plexiform plexiform lesion and neointimal proliferation), which also obstruct the pulmonary arteries and arterioles. The plexiform lesion is observed in approximately 15% of PAH patients and is a pathological hallmark of IPAH and is not as com-

mon in the other types of PAH. Plexiform lesions arise from the monoclonal proliferation of endothelial cells; migration and proliferation of smooth muscle cells; and the accumulation of circulating inflammatory and progenitor cells[117,120].

Atherosclerosis is a major health concern in the developed world and an increasing burden to emerging economies. The atherosclerotic lesion develops progressively. Endothelial cell damage promotes the recruitment of monocytes which accumulate lipid in the evolving lesion, becoming foam cells. Progression of these early fatty streaks into mature plaques involves the extensive accumulation of inflammatory cells and the coalescence of lipid to form a lipid-rich necrotic core. Smooth muscle cells and fibroblasts enshroud the lipid core, forming a fibrous cap as part of the inherent vascular reparative process[121,122]. Although composed of a complex mixture of extracellular matrix proteins, the collagens, notably type I, constitute the major component (-60%) of total plaque protein[123]. Indeed, gene expression profiling of mouse atherosclerotic lesions reveals that the most over-expressed genes associated with the neointima encode for matrix proteins (collagens type I and III and proteoglycans) as well as matrix-inducing proteins such as CCN2[124]. Collagen type I in the shoulder region or fibrous cap of the plaque is of primary importance in stabilising the plaque and protecting against plaque rupture[125-127]. Collagen not only confers mechanical strength and a structural framework, maintaining plaque integrity, but also influences macrophage activity and smooth muscle cell migration and proliferation. Furthermore, when deposited and assembled into the extracellular matrix, collagen (along with other matrix proteins) functions as an extracellular reservoir for cytokines and growth factors including TGF-β, that rapidly mobilised upon injury to 'kick start' the reparative response. Mature collagen type I is subjected to degradation by matrix-modifying enzymes, notably the matrix metalloproteinases[128,129]. It is likely that the balance between collagen synthesis and degradation is of great importance in the maintenance of plaque stability.

Apolipoprotein E deficient mice (*ApoE-/-*) have been used extensively as model of atherosclerosis[130]. When *ApoE-/-* mice are submitted to guidewire-induced femoral artery injury, remodeling occurs that resembles atherosclerotic lesions which can be observed with time[131]. At 4 weeks following injury, there are significant increases in neointima and adventia expansion (***Fig. 1***) with collagen presence as judged by the red stain in picrosirius stain (***Fig. 1A***). Picrosirius[132] staining specifically identifies all collagens with a red color and, polarized light illuminates

collagen fibrils (types I and III) with different colors relating to the size of the fibers. The fibrillar collagens accumulate primarily in the adventitia as judged by the polarized light pictures (*Fig. 1B*). The collagen fibrils in the neointima, where the fibrous cap forms, are not uniformly located in neointimal space. The matrix formed in the adventitia is primarily fibrillar collagen which increases the stiffness of arteries. The synthesis of ECM proceeds, but cannot replace the components in the same fashion as during embryogenesis. During the development of atherosclerosis collagen and multiple ECM components are increased as reviewed previously[133].

TRANSCRIPTIONAL REGULATION OF COLLAGEN

Because of the importance of collagen type I accumulation in fibrotic disease, its transcriptional control has been extensively studied. Collagen type I is formed by two polypeptide chains α1(I) (COL1A1) and α2(I) (COL1A2) that form a triple helix with two α1(I) chain and one α2(I) chain. The genes are on two separate chromosomes and with different promoters, although regulated in coordinately in most cells. Transgenic animals have been used to map tissue specific regions in the two promoters. The *COL1A1* promoter is expressed in vascular smooth muscle using an upstream region with 3.6 kb of the start site, smaller promoters are not expressed in the vasculature[134]. Transgenic mice with the minimal promoter between -350 and +54 has low level expression in all tissues[135]. This expression is enhanced by a far upstream enhancer about 17 kb away from the transcription start site[136] (described in detail below).

The *cis*-acting elements in *COL1A2* with transcriptional regulators of importance for gene expression has been reviewed[137]. The proximal promoter has a cluster of cis-acting sites at the initiation region and a proximal enhancer. There are combinations of transcription factors and co-regulators that target the promoter by signal
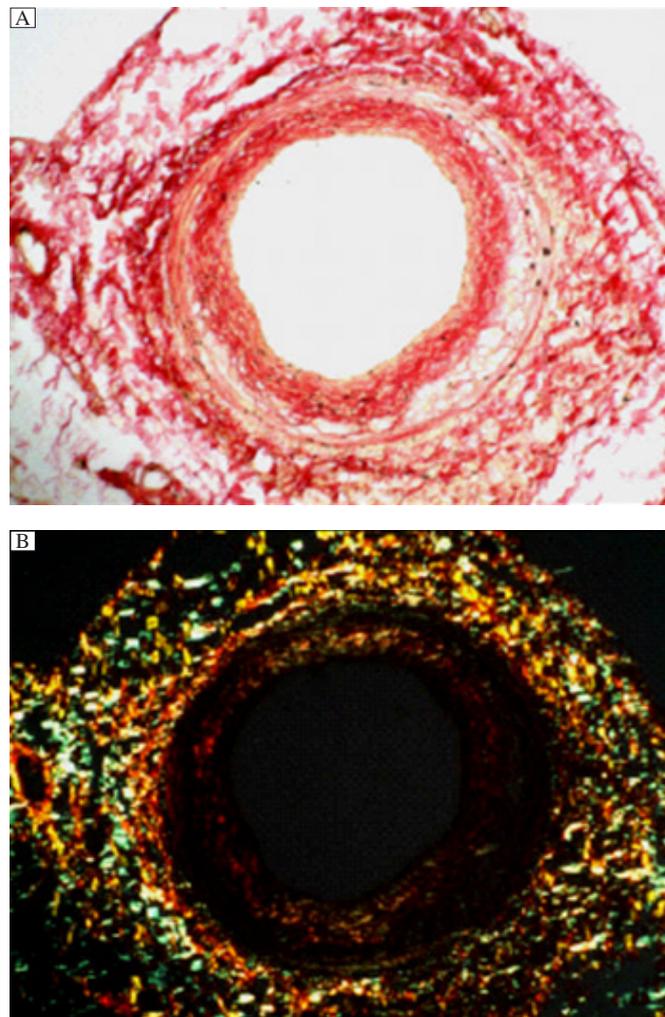

Fig. 1 **Picrosirius Red-stained vascular lesion of *Apoe*-/- mice.** A lesion from a guidewire-injured femoral artery is stained with Picrosirius Red and visualized using light microscopy (A) and polarized light microscopy (B).

transduction pathways both pro-fibrotic and anti-fibrotic cytokines. This review will cover what is new to our understanding of collagen transcription in the vasculature starting with the initiation site region, proximal enhancer and far upstream enhancer.

In mature cells, collagen transcription is repressed in vivo, but poised to be activated during stress or injury. Little is understood about how this is accomplished. One epigenetic method of repression is present at the initiation site of the collagen gene where there is a methylation site (CpG) at +7 within a binding site for a regulatory factor for X box (RFX) family of proteins[138-141]. When the collagen gene is methylated at +7 CpG site within the initiation region, RFX1 binds to and represses collagen transcription by blocking RNA polymerase[140]. During inflammatory responses to injury, interferon gamma (IFNγ) is released into the ECM, stimulating fibroblasts and SMCs. IFN-γ repression of collagen is mediated through an RFX5 complex[142] which recruits CIITA, and represses collagen transcription as well as activating MHC II expression[143]. Both type I genes have an RFX binding site at the transcription start site[144]. Two members of the RFX family, RFX1 and RFX5, associate with distinct sets of co-repressors on the collagen transcription start site. RFX5 specifically interacts with histone deacetylase 2 (HDAC2) and the mammalian transcriptional repressor (Sin3B), whereas RFX1 preferably

interacts with HDAC1 and Sin3A145. CIITA becomes phosphorylated by glycogen synthase kinase 3 (GSK3) and interacts with a transcription repressor complex containing histone deacetylase 2 (HDAC2/Sin3B) to alter chromatin and represses transcription[146,147]. IFN-γ induces CIITA expression in a time dependent manner and promotes CIITA occupancy on the collagen type I genes and MHC II genes[143]. Short hairpin interference RNA (shRNA) against CIITA expressed in lentivirus specifically eliminates the IFN-γ stimulated expression of CIITA, leading to the alleviation of COL1A2 repression and MHC- II activation[143]. In addition, peroxisome proliferator-activated receptor gamma (PPARγ) interacts with CIITA/RFX5 complex to repress type I collagen gene expression[148] and increased cAMP blocks the CIITA repression of collagen[149]. A model of the repression during IFN-γ stimulation is shown in *Fig. 2*.

The proximal enhancer contains multiple active Sp1 sites, a Smad binding site, several Ets sites, and a CCAAT/enhancer-binding protein (C/EBP) as reviewed previously[137]. There are several key positive and negative regulator proteins of collagen transcription in the setting of fibrotic disease that interact with these sites. The Sp1 family of proteins activates collagen transcription through G/C rich sites[150,151], while the Ets domain family of proteins both activate and repress collagen gene expression in fibroblasts[152,153].
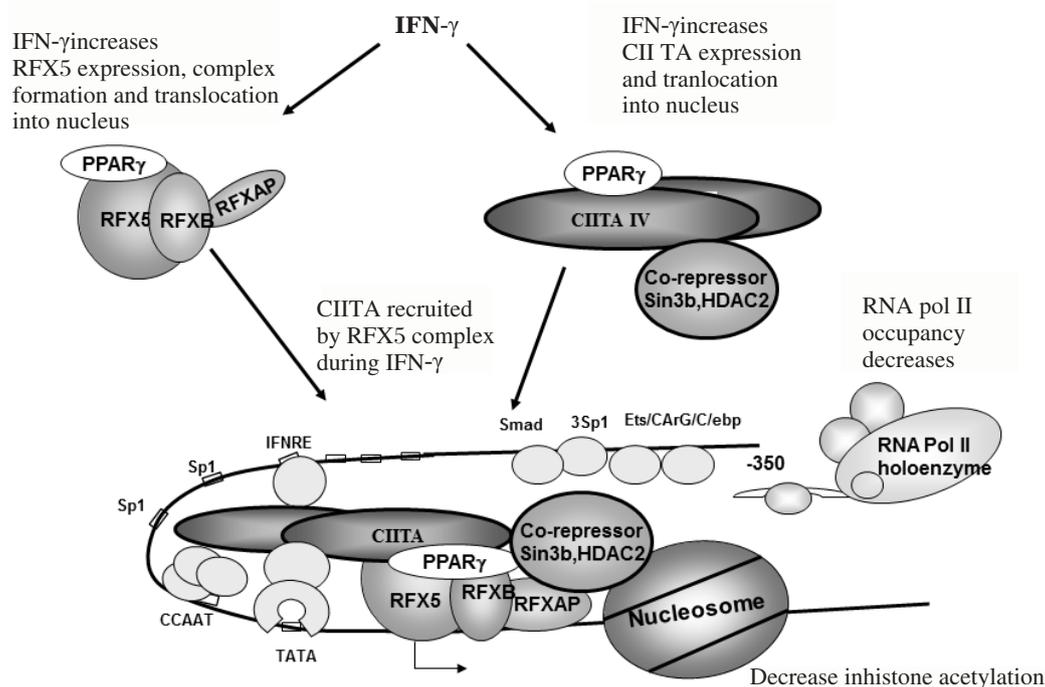

***Fig. 2* Model of collagen repression by CIITA during IFN-γ.** A schematic representation of collagen COL1A2 promoter during IFN-γ treatment.
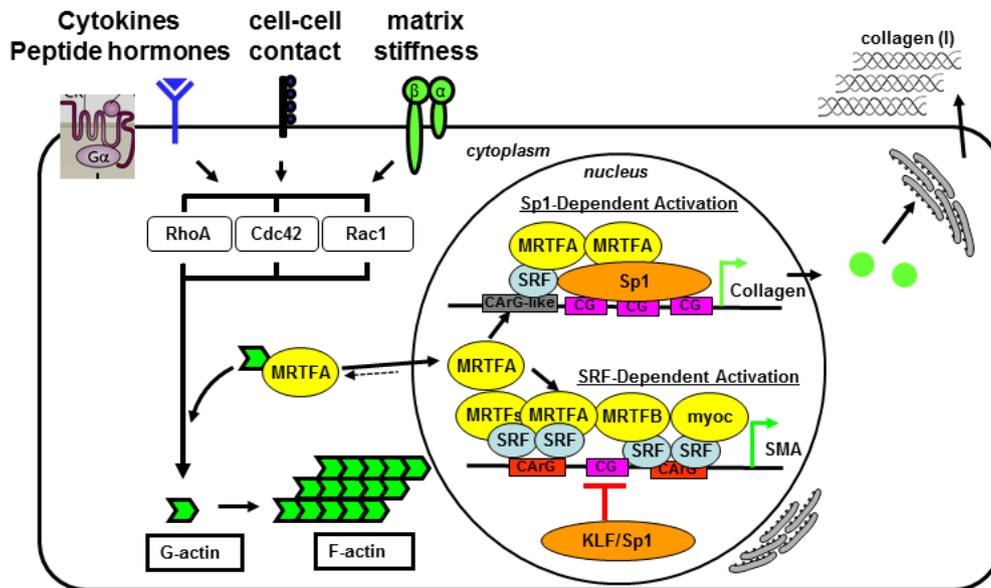
Fig. 3 **Differential activation of collagen and vascular markers (SMA) by myocardin family.** A schematic representation of differential activation of collagen COL1A2 and SMA promoters.

TGFβ activates collagen through Smad binding site with Sp1154 whereas TNFα represses collagen through C/EBP site[155]. *MRTFA* deficient mice have less cardiac fibrosis following myocardial infarction or angiotensin II treatment. Collagen is a direct target of MRTFA signaling through TGFβ and Rho activity[156]. MRTFA is recruited to collagen with SRF and Sp1 which is a strong inducer of gene expression[112]. The SRF CArG-like binding site, which overlaps with the C/EBP and one Ets site, is similar to the degenerate CArG site in the SMA promoter necessary for SMC switching from contractile to synthetic[157]. MRTF-A, an important regulator of collagen synthesis, exhibits a dependence on both SRF and Sp1 function to enhance collagen expression. Sp1 synergizes with MRTFA to increase collagen transcription[112] whereas during SMC phenotypic switch, Sp1 is activated to repress SMA transcription[158]. MRTFA is a stronger activator of collagen transcription than the other family members[112]. Loss of MRTF-A in knockdown and knockout models shows an inhibition of collagen and SMA expression, suggesting a disruption of myofibroblast function. Expression of MRTFA in the knockout cells rescues the decreased collagen synthesis. Therefore, when injury occurs to vasculature and there is a reciprocal increase in MRTFA with a decrease in myocardin and MRT-FB[84] bringing the cells phenotypically closer to myofibroblasts, the stage is set for collagen up-regulation and SMA down regulation (see model *Fig. 3*).

Current knowledge of transcriptional regulation of *COL1A2* in vivo has been obtained from studies us-ing promoter sequences driving transgene expression in mice. Transgenic studies have identified a "far upstream enhancer" located 21.8 to -18.8 kb upstream of the start site of transcription of the *COL1A2* gene[136,159,160]. When the far upstream enhancer region is cloned upstream of the proximal promoter, it directs high levels of transgene expression, significantly in almost all mesenchymal tissues where the endogenous *COL1A2* is expressed[161]. High degree of homology is found between the human and murine enhancers[160]. In addition to the absolute requirement of the enhancer to govern temporal and tissue/cell specific expression of collagen in vivo, using transgenic reporter mice, the presence of the enhancer has also been shown to be essential for the activation and re-activation of collagen type I expression in adult mice during connective tissue remodelling and repair[161], wound healing and in pathological scenarios such as excessive scarring and fibrosis[162-164]. Thus, the enhancer is crucial, conveying both temporal and tissue-specific expression of collagen type I to all mesenchymal cell lineages including fibroblasts, pericytes and vascular smooth muscle cells[161]. Of significant importance in relating to vessel remodelling, a specific sequence motif located within the enhancer region is responsible for the enhanced transcriptional activity and expression of collagen type I in blood vessels and in particular vascular smooth muscle cells only. This element was essential for the correct expression of collagen type I in vivo in vas−cular smooth muscle cells. Furthermore, this mo-tif is recognised by the transcription factor, Nkx2-5, which

binds to and activates collagen gene transcription, as part of an activation/repression system with the tran–scription factor δEF1 (Zeb1).

CONCLUSION

The vascular system consists of specialized cells surrounded by a dynamic ECM that not only provides structure through connections of cells within the network, but also instructs cellular function. ECM components sequester growth factors and make them available to cells. The ECM is also necessary for providing mechanical signals that result in cell responses including synthesis of ECM, migration, proliferation and apoptosis. Cells respond to ECM by remodeling their microenvironment which becomes dysregulated in disease.

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