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Smooth Muscle and Extracellular Matrix Interactions in Health and Disease

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Abstract

Alterations in smooth muscle cell function and phenotype contribute to tissue remodeling in various pathologies including obstructive lung (*e.g.*, asthma) and vascular (*e.g.*, atherosclerosis) diseases. The extracellular matrix (ECM) is a major influence on the biology of smooth muscle cells, being an important support structure that provides signaling cues through its biochemical and biophysical properties. ECM factors activate biochemical and mechano-transduction signaling pathways, which modulate smooth muscle cell contraction, stiffness, survival, growth, cytokine production and migration (*i.e.*, cellular processes which contribute to changes in tissue architecture). The interaction of the ECM with smooth muscle cells is a dynamic multi-directional process, as smooth muscle cells also produce ECM protein, as well as proteases and cross-linking enzymes which regulate ECM form and structure. Understanding the molecular basis of ECM modifications and their impact on smooth muscle cell function in disease may lead to the development of novel therapies. This chapter reviews interactions between the ECM and smooth muscle cell and how they become altered in disease, using obstructive lung and vascular diseases as examples. From a pharmacological and therapeutic perspective, strategies that alter the phenotype of the smooth muscle cell in disease will be discussed. Emphasis will be given to approaches that target the proteases and mediators of ECM-smooth muscle cell signaling as potential treatments for pulmonary and vascular disease. Proteases of the coagulation and plasminogen activation systems have been given particular attention as they not only have a role in forming and modifying ECM, but also can directly stimulate changes in smooth muscle cell function and phenotype via activating receptors such as the protease-activated receptor-1 (PAR-1) and integrins.

1. Introduction

Smooth muscle cells function by contracting following activation of actin and myosin filaments, in a process involving myosin light chain phosphorylation, mediated by Ca^{2+}-dependent pathways [1]. In many diseases, alterations in smooth muscle cell function including contractile responses, growth and phenotype, contribute to tissue remodeling. In obstructive lung diseases such as severe asthma and chronic obstructive pulmonary disease (COPD), increases in the stiffness and mass of the airway smooth muscle (ASM) bundle contribute to fixed airway obstruction and hyper-responsiveness [2, 3]. In vascular injury and diseases such as atherosclerosis and pulmonary arterial hypertension (PAH), the migration, stiffening, proliferation and growth of vascular smooth muscle (VSM) cells contribute to the enlargement of the blood vessel wall, which in effect reduces lumen size, thus an increase in vascular resistance [4, 5]. Alterations in the microenvironment of the smooth muscle cell, particularly in the composition and structure of the ECM, accompany changes in smooth muscle biology in disease. Smooth muscle cells have a large role in modifying their microenvironment in disease by producing ECM protein (*e.g.,* collagen, fibrin, fibronectin and proteoglycans [6]) and factors which regulate ECM formation (*e.g.,* tissue factor in fibrin formation [7]). Furthermore, smooth muscle cells secrete proteases (*e.g.,* urokinase plasminogen activator or uPA [8]) and crosslinking enzymes (*e.g.,* lysyl oxidases [9]) which modulate ECM structure and form. The ECM in turn regulates smooth muscle function by the provision of both biochemical and biomechanical cues, in a process involving complexes formed between integrins, focal adhesion (FA) proteins and the actin-cytoskeleton. Both biochemical and mechano-transduction signaling in smooth muscle cells are mutually interdependent. In disease, the altered ECM may perpetuate tissue remodeling by augmenting smooth muscle growth, migration, cytokine production, cell stiffness and proliferation in a detrimental feed forward mechanism. Aside from important biomechanical contributions in tissue remodelling, smooth muscle cells are also potent producers of an array of inflammatory mediators, including cytokines, chemokines and cell adhesion molecules (CAMs) [10-13]. These inflammatory mediators, as well as the ECM produced by smooth muscle cells, influence the type and quantity of inflammatory cells that infiltrate damaged tissue in disease [14, 15].

2. Smooth muscle cells

Smooth muscle cells are phenotypically-plastic stromal cells, which are very capable of differentiating in response to injury and inflammation in disease. Whilst myogenic, the structure, mechanical properties, contractility and function of smooth muscle cells are different

to those of striated and cardiac muscle cells. The involuntary non-striated smooth muscle cells are found in many tissues and organs including the gastro-intestinal tract, the respiratory system, reproductive tract, urinary bladder, skin, iris of the eyes, kidneys and blood vessels. Smooth muscle cells contract and relax to regulate the luminal diameter and viscoelasticity of conducting vessels (*e.g.*, the vasculature and bronchioles) [16] and sphincters (*e.g.*, the urethral and pre-capillary sphincters) [17]. Smooth muscle contraction also has a role in the rhythmic peristalsis of tissues and organs of the gastro-intestinal tract and respiratory systems [18]. Whilst the structure and function of smooth muscle cells in different tissues are quite similar, they can contrast in their mode of activation, whether that be spontaneous involving ionic channel dynamics or by physiochemical agents (*e.g.*, hormones and neurotransmitters) or external agents (*e.g.*, CO_2). The actin-myosin contractile apparatus mediates the force generation responsible for smooth muscle contraction, whether that contraction is phasic or tonic (*i.e.*, rapid versus sustained contraction) [19]. Contraction is initiated by calcium-regulated myosin light chain phosphorylation, involving calmodulin rather than the calcium-activated troponin system (as in striated and cardiac muscle), and involves the sliding of actin-myosin filaments [1].

3. Smooth muscle cells in disease

Structural and functional changes to smooth muscle cells and their microenvironment contributes to tissue remodeling in diseases such as lung obstructive and vascular diseases [2-5], which are the focus of this chapter. Tissue remodeling involving smooth muscle cells is likely to be a result of injury and dys-regulated repair processes linked to inflammation and extravascular coagulation and fibrinolysis [20-23].

3.1. Obstructive lung diseases

Obstructive lung diseases, including asthma, COPD, bronchitis and bronchiectasis, are characterized by airway obstruction. The latter is a limitation of airflow, caused by the narrowing of bronchioles. The mechano-contractile properties of the ASM cell make it the primary effecter of bronchospasm, which is an acute contraction of the airways that occurs in asthma and bronchitis. ASM cells are also involved in the array of persistent tissue structural changes of the airway wall that contribute to airway obstruction in asthma and COPD [2, 24, 25]. In airway wall remodeling (AWR), ASM cell hyperplasia, hypertrophy and changes in production of ECM protein contribute to an increase in airway wall thickness and reduction in airway wall distensibility [26]. ASM cells in disease are major contributors to the increases in production of ECM components, including the wound type collagens, I and III, and fibronectin. ASM cells also have an important inflammatory role in airway obstructive diseases, being potent producers of growth factors, cytokines and other pro-inflammatory mediators, including granulocyte macrophage-colony stimulating factor (GM-CSF), intracellular adhesion molecule (ICAM), interleukin-1α (IL-1 α), eotaxin, leukaemia inhibitory (LIF), fractalkine and vascular cell adhesion molecule (VCAM) [10]. Pro-inflammatory mediator expression in ASM cells is stimulated primarily by cytokines and growth factors produced by

inflammatory cells and the epithelium [27]. The ECM in the microenvironment of the ASM cell can modulate cytokine expression by ASM cells [28]. Furthermore, cyclical changes in mechanical strain associated with breathing also modulate cytokine expression by ASM cells [29]. As a consequence of their pivotal role in AWR, therapies that target the ASM cell are continually being explored as possible new treatments for obstructive lung diseases [30]. Although eliminating ASM by bronchial thermoplasty is effective in reducing airway obstruction, less invasive and costly therapies are urgently needed. An effective muscle relaxing, anti-remodeling treatment that specifically targets ASM cells is expected to reduce airway reactivity and symptoms in patients with asthma or COPD [31, 32].

3.2. Vascular diseases

VSM cells reside in the medial layer of blood vessels, mediating changes in vascular tone by contracting or relaxing in response to vasoconstrictors and vasodilators respectively. In vascular injury and disease, VSM cells switch from a quiescent, contractile phenotype to a synthetic phenotype, which produces cytokines and ECM protein (*e.g.,* fibronectin and sulphated proteoglycans), undergoes hyperplasia (*i.e.,* increased proliferation) and/or hypertrophy (*i.e.,* increased cell size). In atherosclerosis, a cause of stenosis and blood vessel occlusion, synthetic VSM cells migrate from the media to the intima where they proliferate and contribute to intimal thickening [22, 23]. Furthermore, within the intima, VSM can alter the composition of the ECM. For example, VSM cells produce increased amounts of LDL-accumulating sulphated proteoglycans, which increase lipid loading, a feature of athero-thrombotic disease [33]. In hypertension, VSM cells undergo hypertrophic remodeling in the media of blood vessels, contributing to excessive arterial vascular contractile tone, hence high blood pressure [34]. Hypoxia, inflammation and shear stress contribute to vasoconstriction and pulmonary vascular remodeling in PAH [35, 36]. Medial thickening in proximal pulmonary vessels is caused by the hypertrophy, hyperplasia and ECM production of resident VSM cells, whereas in pre-capillary arterioles, the VSM cells that contribute to medial thickening are derived from intermediate cells in blood vessels or adventitial fibroblasts, which differentiate into VSM. Like ASM cells, VSMs contribute to inflammation in vascular disease by producing pro-inflammatory mediators such as the platelet-derived growth factor (PDGF), transforming growth factor-β (TGF-β), interferon-γ (IFN-γ), and monocyte chemotactic protein-1 (MCP-1). Furthermore, VSM cells express CAMs (*e.g.,* VCAM-1 and ICAM-1), which have a role in retaining monocytes and macrophages within lesions such as those formed in the intima in atherosclerosis. Aberrant Akt/PI3kinase signaling resulting from a defect in PTEN phosphatase activity is thought to contribute to the pro-inflammatory phenotype of VSM cells in PAH [37]. In a murine model of PAH, the targeting of the PTEN gene specifically in VSM cells augments an inflammatory response and vascular remodeling [38].

4. ECM of smooth muscle

The ECM of the smooth muscle cell microenvironment changes in injury, disease and with ageing. ECM production and modification by smooth muscle cell is regulated by intrinsic

(*e.g.,* epigenetics) and extrinsic (*e.g.,* oxidative stress, growth factors and cytokines) factors [15]. Abnormalities of the ECM are a key feature of tissue remodeling in obstructive lung [39] and vascular disease [34].

4.1. Collagen

Collagen is the most prominent structural protein of connective tissue. Native collagen I and III form fibrils comprising supra-molecular aggregates of collagen that are stabilized by interactions between their helical domains [40]. These fibrils have a high tensile strength that maintains the structural integrity of tissue by counter-balancing distending forces, such as those evoked by breathing on the airway wall [41]. In the airways of patients with asthma and COPD, the ECM is expanded by fibrils of collagen I and III, including around and within smooth muscle bundles, reducing airway wall distensibility [26]. Increased collagen, as well as ASM hyperplasia and hypertrophy, also contribute to airway wall thickening that is linked to increased airway-reactivity [2]. In the media of the healthy blood vessel wall, collagen I and III are the main components of the ECM, with elastin also being abundant in the media of larger arterioles [42, 43]. Collagen fibrils formed by VSM cells also have an important role in fibrous cap stabilization, a stage of atherogenesis [44]. Whilst the native forms of collagen impose mechanical strain on cells, it is the denatured forms of collagen, which perhaps are more biochemically-reactive. Increases in the activities of plasmin and MMPs, proteases which denature native collagen, occur in both respiratory [45] and vascular [34] disease. The denaturation of collagen fibrils immediately adjacent to and surrounding smooth muscle cells by pericellular proteolysis may have an important role in regulating phenotype and function in disease.

4.2. Fibronectin

Fibronectin is a high molecular weight glycoprotein and constituent of the ECM. Fibronectin binds to the $\alpha5\beta1$ integrin on cells to transmit biochemical and mechanical signals, and is a nucleator of fibrillogenesis (*i.e.,* collagen fibril assembly). In asthma, levels of fibronectin, along with collagen I and III, are increased within the smooth muscle bundle of the airway wall [46]. Additionally, ASM cells from asthmatics, when compared to non-asthmatics, secrete more fibronectin, which in turn augments increased ASM cell proliferation and cytokine production [47]. Rhinovirus-infection [48], cigarette smoke extract [49] and the pro-fibrogenic mediator TGF-β [50], all stimulate ASM cells to produce fibronectin. VSM cells in disease also produce increased amounts of fibronectin. During atherogenesis, fibronectin synthesized by VSM cells becomes more abundant in the blood vessel wall, which augments fibrillogenesis [44] and stimulates smooth muscle cell proliferation and cytokine production [42, 43].

4.3. Fibrin

Extravascular accumulation of fibrin, formed by the coagulation cascade, occurs in a number of diseases, including respiratory and vascular diseases [21, 51-55]. Increased vascular permeability allows blood-circulating hemostatic factors such as factor VII (FVII), factor X (FX)

and plasminogen to enter damaged and inflamed tissue to become activated and participate in coagulation and fibrinolysis (Figure 1).
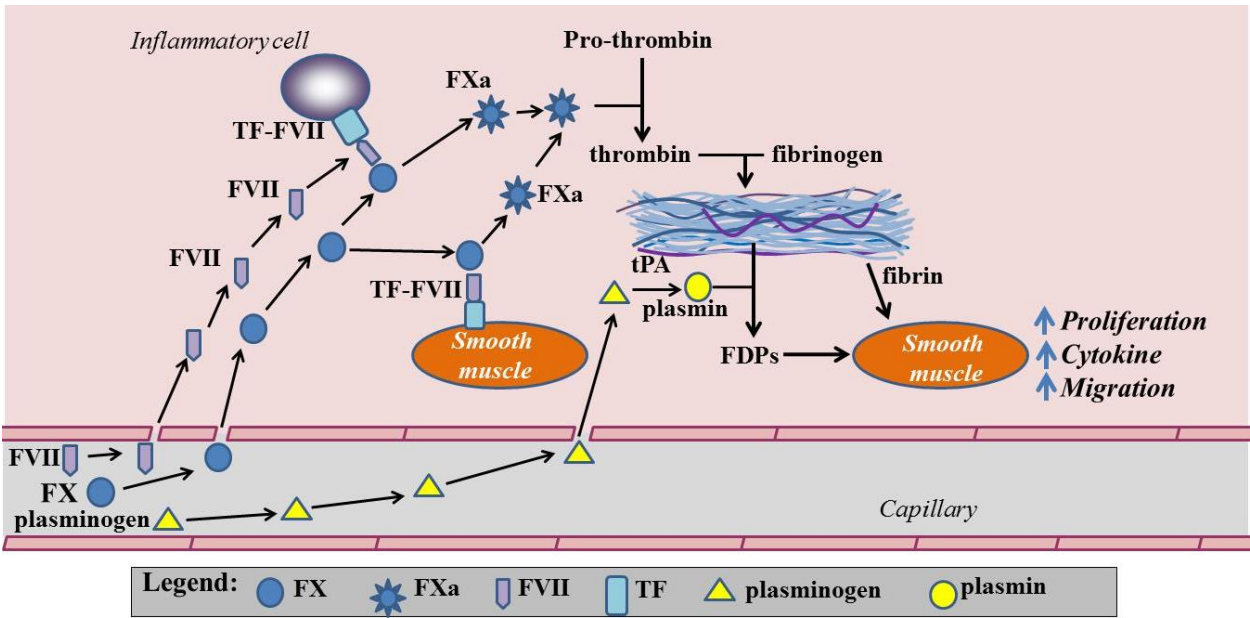

Figure 1. Extravascular coagulation and fibrinolysis. In inflamed tissue such as the airway wall in asthma, plasma containing factor VII (FVII) and FX leak into the extravascular compartment. FVII, combined with tissue factor (TF), formed by epithelial, stromal (e.g., smooth muscle cells) and inflammatory cells, transforms FX into the serine protease, FXa. The latter, combined with FV, activates thrombin, which in turn converts fibrinogen into fibrin. Whilst fibrin has an important role in physiological wound-repair, it also serves as a scaffold for migrating stromal cells, and can be converted into fibrin degradation products (FDPs), which stimulate smooth muscle cell migration and cytokine production. Fibrinolysis (i.e., the break-down of fibrin) is catalysed by plasmin, formed by the activation of plasma-derived plasminogen. The latter is catalysed by tissue-type plasminogen activator (tPA).

Abundant extravascular fibrin is a specific hallmark of lung disease including asthma, and is thought to be a result of hyper-coagulation and suppressed fibrinolysis (*i.e.*, fibrin degradation). The key mediator of fibrinolysis is plasmin, which can be formed by the proteolytic activation of plasminogen by either the tissue-type or urokinase type plasminogen activators (tPA or uPA respectively) [56, 57]. However, only tPA has fibrin-dependent amplification activity, facilitating its role as the primary mediator of fibrinolysis. Levels of tPA are much higher than uPA in the airway lumen [58-60]. The reduced AWR observed in plasminogen activator inhibitor-1 (PAI-1) gene knockout mice challenged with aerosolized allergen is likely to be a consequence of less airway fibrin [61, 62]. PAI-1 inhibits fibrinolysis by blocking the actions tPA in plasmin formation.

The medial layer of blood vessels, where VSM cells are prominent in number, is exposed to plasma exudate, even under normal physiological conditions. Whilst fibrin is present in normal arterial intima, its levels are increased in atherosclerotic lesions, particularly early proliferative, gelatinous-lesions [21]. Again, hypercoaulation and suppressed fibrinolysis are likely to contribute to increased fibrin formation in the vasculature in disease. The coagulant, tissue factor (TF), is highly expressed in VSM cells during atherogenesis [7], being rapidly induced by growth factors and cytokines [7]. Hypoxia, a driver of PAH, induces the up-

regulation of PAI-1 in pulmonary artery VSM cells [63]. In injury and disease, the deposition of fibrin into the ECM serves as a scaffold to support smooth muscle cell migration [64]. Furthermore, fibrin degradation products (FDPs) formed by fibrinolysis, stimulate production of pro-inflammatory mediators (*e.g.*, C-reactive protein in VSM cells [65]).

4.4. Proteolglycans

Proteoglycans are another important component of the ECM synthesized by smooth muscle cells in disease including VSM cells during atherogenesis [42, 43] and ASM cells in asthma [66]. In atherogenesis, sulphated proteoglycans, via ionic interactions with ApoB100 and ApoE, entrap low-density lipoprotein (LDL) within the vessel wall. Bound oxidized-LDL augments macrophage lipid uptake and foam cell formation, and stimulates VSM cells to secrete greater amounts of sulphated proteoglycans [34].

5. ECM remodeling by smooth muscle cells

Asides from producing ECM protein, smooth muscle cells also modulate the structure and form of the ECM. Extracellular modification of ECM protein involves integrins and enzymes which proteolyze and cross-link collagen. The serine protease, plasmin, also has the ability to directly activate smooth muscle cells via receptor based mechanisms. Major modifications of ECM protein by smooth muscle cells are described below:

5.1. Fibrillogenesis

Smooth muscle cells including VSM [67] and ASM [68] cells polymerize collagen fibrils into larger supramolecular collagen assemblies. Collagen fibrils can vary greatly in diameter (20-500 nm) and form different supramolecular structures such as bundles, weaves, and layers to suit differing roles and functions. The ability of smooth muscle cells to increase the diameter of fibrils would increase the tensile strength and resistance of the fibrils to collagenolysis by MMPs. Collagen fibrillogenesis involves integrin binding (*e.g.*, $\alpha2\beta1$, $\alpha11\beta1$) and is integrated with fibronectin fibril formation [69]. Lipid accumulation by VSM cells inhibits collagen fibrillogenesis [70], contributing to the destabilization of fibrous caps, hence rupture of atherosclerotic plaques [44].

5.2. Proteolysis

Matrix metalloproteases (MMPs) and plasmin are proteases involved in the degradation of ECM proteins such as fibronectin and denatured collagen. Plasmin also cleaves and activates the zymogen forms of MMPs including the MMP collagenases (MMP-1, MMP-13 and MMP-14), which denature collagen fibrils [71]. MMPs including MMP-1, MMP-2, MMP-12 and MMP-14 are expressed by human ASM cells *in vivo* [72] and/or *in vitro* [68] and conversion of plasminogen into plasmin by ASM cells is associated with MMP activation and increases in collageneolytic activity [73]. MMPs and components of the plasminogen activation system are

co-expressed during remodeling in the airways following allergen challenge [74]. VSM cells also produce and secrete MMPs including MMP-2, MMP-3, MMP-7 and MMP-9 [34]. MMPs formed by VSM cells are considered to contribute to the thinning, thus destabilization of the atherosclerotic fibrous cap in atherogenesis [44]. Like ASM cells, VSM cells do not produce plasminogen *de novo*, but express urokinase plasminogen activator (uPA), which readily activates plasminogen convected from plasma through the blood vessel wall [22]. Hypoxia induces uPA expression in VSM cells, in association with increased cell migration and matrix invasion, suggesting a role of plasmin proteolysis in PAH [8].

Interestingly uPA and plasminogen gene deletion (but not tPA gene deletion) also reduces hypoxia-induced PAH and pulmonary vascular remodeling in mice [75]. These effects are likely to be independent of fibrinolysis, as uPA does not bind fibrin with the same high affinity as tPA. Plasmin formed by uPA has pro-inflammatory and -remodeling activities which are not associated with the break-down of fibrin. Furthermore, uPA has plasmin(ogen)-independent effects. The amino-terminal fragment of uPA interacts with its receptor, uPAR to activate other receptors such as the formyl-peptide receptor 2 (FPR2) [76] and the epidermal growth factor receptor (EGFR) [77], to regulate migration, chemotaxis and cytokine production. uPA binding also regulates the affinity of uPAR for the $\alpha3\beta1$-integrin [78], to regulate cell adhesion and cell signaling. Furthermore, the kringle domain of uPA interacts with the $\alpha v\beta1$-integrin in an uPAR-independent manner [79]. Increases in the levels of uPA are associated with a number of pathologies, including asthma, COPD and PAH [59, 60, 80-82]. For smooth muscle cells, plasminogen activation by uPA is accelerated by the annexin A2 hetero-tetramer (AIIt) [83], an extracellular protein complex comprised of annexin A2 and S100A10 (p11). The AIIt also serves as a signal transducer for plasmin in mediating its pro-inflammatory effects on ASM cells [84] and macrophages [85]. The importance of annexin A2 in cancer is becoming increasingly recognised, however, little is still known about the role of annexin A2 in respiratory disease [86-90].

The proteolytic activity of plasmin releases the otherwise latent forms of growth factors such as epidermal growth factor (EGF) and TGF-β [91, 92]. Plasminogen activation by smooth muscle cells is associated with MMP activation [73] and targeting the EGF-receptor (EGFR) or MMPs attenuates plasmin(ogen)-stimulated proliferation [83]. The effects of plasmin(ogen) on EGFR signaling are contributed by heparan sulphate proteoglycan - binding EGF (HB-EGF), an EGFR ligand, which is released from heparin by MMP-mediated proteolysis (Figure 2). Like EGFR transactivation, plasmin-stimulated mobilization of matrix-bound TGF-β contributes to collagen synthesis in smooth muscle cells in a manner involving TGF-β receptor signaling [91]. TGF-β also stimulates ASM cell proliferation [93], in an indirect manner involving autocrine bFGF production [94].

Plasmin also activates the protease-activated receptor-1 (PAR-1), the proto-typical receptor of thrombin (and FXa). PAR-1 is expressed in inflammatory cells including, macrophages, mast cells and eosinophils [96-99], and extravascular structural cells including, smooth muscle cells [100]. Activation of PAR-1 in stromal cells is considered to evoke pro-tissue remodeling activities, which contributes to disease pathology. Levels of PARs are increased in structural cells in tissue remodeling lung diseases [101], and targeting PAR-1 reduces pulmonary
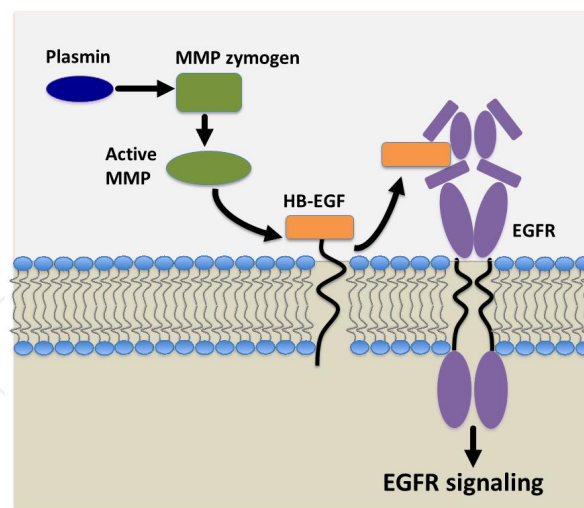
Figure 2. Plasmin-evoked EGFR transactivation. HB-EGF is an important ligand of EGFR, and is released from heparan sulphate proteoglycan by the proteolytic actions of MMPs. HB-EGF is expressed in the airway epithelium and smooth muscle in situ [95].

inflammation and tissue remodeling in mouse models of lung injury and disease, including asthma [98, 102, 103]. Furthermore, PAR-1 activation elicits increased cytokine and collagen expression [103-105] and proliferation [106] of lung stromal cells, including ASM cells [107]. The up-regulation of both PAR-1 and PAR-2 in VSM cells following injury and their subsequent activation by hemostatic proteases is considered to contribute to the pathogenesis of atherosclerosis [5]. Whilst plasmin has ~10 times less affinity for PAR-1 than thrombin [108], integrin co-receptors augment plasmin-evoked PAR-1 activation [109]. Binding to $\alpha_9\beta_1$ integrin localizes plasmin to the cell surface and protects it from α2-antiplasmin inhibition, increasing PAR-1 activation, whilst also activating pathways downstream of $\alpha_9\beta_1$ integrin through integrin-linked kinase (ILK) [109]. Additionally to plasmin, the plasmin-activated MMP-1 and MMP-13 also cleave the N-terminal exodomain of PAR-1, but at sites alternative to those of thrombin, FXa and plasmin, eliciting distinct cellular responses [110].

5.3. Cross-linking

Dysregulated matrix cross-linking and stability contributes to tissue remodeling in vascular disease and ageing. Lysyl oxidases cross-link collagen fibrils by modification of the ε-amino group in the side chain of lysines. Lysyl oxidase activity is increased in the vascular lesions of patients with PAH [9]. The expression of lysyl oxidases in VSM cells is responsive to hypoxia and increases in the lungs of mice in experimental PAH [9]. Glycation of collagens also induces covalent bridges between collagen fibrils, an age-related modification that contributes to arterial stiffness [34]. Similarly, vascular calcification, which involves transglutaminase-2-induced collagen crosslinking and dimerization of osteopontin, is a degenerative and end-stage process of atherosclerosis, which also contributes to the stiffness of ageing arteries [111]. Vascular calcification involves the de-differentiation of the VSM cell to a pro-calcificatory phenotype, where expression of the matrix Gla protein (MGP) and bone morphogenetic protein-2 (BMP-2) are decreased and increased respectively.

6. Smooth muscle cell and ECM interactions

Smooth muscle cells are highly sensitive to the biochemical and mechanical state of the surrounding ECM. Signal transduction, and the transmission and distribution of mechanical forces within the cell are dependent on the actin-cytoskeleton. This deformable polymer network also has important roles in maintaining cell shape (size) and cell migration [112]. The interaction of the actin-cytoskeleton with the contractile apparatus and the ECM involves integrins and focal adhesion (FA) complexes, and is a key determinant of the biochemical and mechano-sensing properties of smooth muscle cells. The highly malleable actin-cytoskeleton constantly rearranges, in a process involving assembly and disassembly, in response to both bio-physical and -chemical stimuli [113]. Alterations in smooth muscle stiffness in health and disease involve changes to actin-cytoskeleton organization, which is influenced by the ECM [114].

6.1. Biochemical transduction

Biochemically, ECM protein signal through integrin receptors and the dystrophin-glycoprotein complex in smooth muscle cells [115]. The native fibrillar and denatured non-fibrillar forms of collagen regulate smooth muscle cell function differentially. The anti-proliferative effect of fibrillar collagen type I on VSM [116] and ASM [68] cells, which arrest cells in the G1 phase of the cell cycle, involves $\alpha2\beta1$ integrin binding. Although, the non-fibrillar forms of collagen types I and III do not contribute structurally to tissue integrity, they retain cell signaling activity. The GFOGER motif of the characteristic triple helix of non-fibrillar type I collagen binds with high affinity to the integrins $\alpha1\beta1$, $\alpha2\beta1$, $\alpha10\beta1$, and $\alpha11\beta1$ [117]. Furthermore, proteolytic cleavage of the helix by MMPs and plasmin reveals the 'matricryptic' RGD integrin-binding site [118]. Non-fibrillar type I collagen stimulates smooth muscle cell proliferation [119], survival [120], and cytokine release [28] in an integrin-dependent manner. Similarly, fibronectin, which binds $\alpha5\beta1$ integrin via its RGD motif, also stimulates smooth muscle cell proliferation [119] and augments cytokine production in response to stimuli such as IL-1β [28]. Fibrin degradation products (FDPs) also regulate smooth muscle cell migration via binding the $\alpha_5\beta_3$ integrin [64], as well as stimulating smooth muscle cell cytokine production and proliferation via binding TLR-4 [121].

6.2. Mechano-transduction

Connections between the ECM and the actin cytoskeleton of smooth muscle cells allows for an efficient transfer of force between the contractile apparatus and the extracellular environment, which is important in myogenic force generation [122]. These same connections, which involve integrins and FA complexes, also regulate smooth muscle cell stiffness and phenotype. Mechanical forces, whether external or internal to tissue in which smooth muscle cells reside, stretch and strain protein-cell surface integrin FA complexes, activating downstream intracellular signaling pathways. This process is termed mechano-transduction, and often elicits mechanically sensitive ion channels to open or close, changing the polarity of the membrane potential, which then activates voltage-gated channels. Mechanical strain in smooth muscle

cells activates RhoA, a pivotal regulator of actin adhesion organization [123]. The β1-integrin is an important mechanoreceptor, regulating cell function and viability via the inositol 3-kinase (PI3K)/Akt signaling pathway, in a process involving FA kinase (FAK) and integrin-linked kinase (ILK) phosphorylation [124, 125].

In arterial blood vessels, integrin-mediated mechano-transduction triggers intracellular Ca^{2+} mobilization in VSM cells to evoke myogenic responses [126]. The integrin-mediated adhesion of VSM cells to the ECM is dynamically regulated in opposing directions by vasoconstrictors (*e.g.,* angiotensin II) and vasodilators (*e.g.,* nitric oxide) to control cell contractility, hence blood vessel tone [127]. VSM cells are in turn subject to varying mechanical strain (*i.e.,* intraluminal pressure) generated by moving blood. In the airways, the ASM cell regulates bronchomotor tone, contracting in response to various contractile agonists including acetylcholine, serotonin, histamine and endothelin-1 [128]. The ASM cell is also subject to mechanical forces resulting from cyclical expansion of airway diameter and cyclical lengthening of the airway wall due to breathing [122]. The ECM modulates the effects of these forces on cell function and phenotype. Cyclical strain (4% elongation) that is equivalent to normal breathing inhibits ASM cell proliferation when grown on laminin, but not collagen type I (denatured) *in* vitro [129]. The ratio of laminin to collagen is higher in the airways of non-asthmatics than asthmatics. When ASM cells grown on type I collagen are subject to increased cyclical strain (17-18% elongation), equivalent to those generated by bronchospasm and hyperinflation, an increase in migration and proliferation occurs in a MMP-dependent manner [130].

In disease, smooth muscle cell biology is affected by changes in the ECM, including increases in substrate stiffness and the generation of isometric forces in tethered collagen lattices, which influence mechano-transduction. In severe asthma, the "strait jacket" effect of ASM cells being embedded in a rigid microenvironment may reduce the amplitude of the oscillatory forces associated with breathing. This is likely to have potential consequences on smooth muscle biology, as has been proposed for lung fibroblasts in fibrotic foci (FF) lesions in idiopathic pulmonary fibrosis (IPF) [131]. Tensile collagen fibrils within FF form a rigid environment that reduces the magnitude of cyclical strain the fibroblasts are normally subjected to in healthy tissue upon breathing. A reduction in the amplitude of force of breathing-associated cyclical strain is thought to increase lung fibroblast fibrogenic activity and stiffness.

6.3. Interconnection of biochemical- and mechanical-evoked signaling

ECM biochemistry and mechanics are very much interconnected in their regulation of smooth muscle behavior. The influence of mechanics on smooth muscle cell biology is modulated by ligand biochemistry. For example, smooth muscle cells grown on fibronectin, as compared to laminin, exhibit increased cell adhesion and cytoskeletal polymerization in response to increasing stiffness of the substrata, *i.e.,* the 'stiffness by ligand' effect [132]. In VSM cells, FAs formed by cell contact with different ECM proteins, exhibit different mechanical characteristics resulting in distinct force-generating reactions [133]. VSM cells attach more strongly to fibronectin than collagen type I, vitronectin and laminin, to evoke greater myogenic force-generation [133]. Integrin co-receptors may also modulate PAR-1 and uPA signaling in response to biomechanical forces. uPA elicits cellular responses via binding its receptor, uPAR,

which lacks a transmembrane or intracellular domain [78]. The interaction of uPA with uPAR to activate co-receptors including integrins, is likely to be influenced by the engagement of integrins with the ECM. In support, fibulin 5 [127] and vitronectin [41] are integrin binding ECM proteins, which modulate uPA-uPAR signaling [134]. uPAR-independent activation of $\alpha v\beta 1$-integrin by uPA in smooth muscle cell may also be similarly affected by the ECM and biomechanical forces [79].

7. Targeting smooth muscle cell biology as therapy

Strategies that target smooth muscle cell biology, including ECM-smooth muscle cell interactions, may be beneficial in the treatment of tissue remodeling diseases. Approaches that target proteases, which modulate the ECM or ECM-signaling of smooth muscle cells in disease, will be of particular focus in this chapter section.

7.1. Contractile, hypertrophic and hyperplasic regulators

Targeting factors which regulate smooth muscle contractility, hyperplasia and/or hypertrophy are currently used in the treatment of airway and vascular diseases. β_2-Adrenergic receptor agonists, which increase the intracellular second messenger, cyclic AMP (cAMP), in ASM cells, are used pharmacologically to dampen bronchoconstriction in asthma and COPD [135]. Blockade of endothelin (ET) receptors are an effective treatment for PAH. Endothelin-1 (ET-1) is both a vasoconstrictor and mitogen, evoking its effects on VSM cells through binding to ET_A or ET_B receptors. Another treatment of PAH is prostacyclin (or its analogs), an eicosanoid, which like the prostaglandins and thromboxane, is vasodilatory [136]. Eicosanoids, which are released by endothelial cells, stimulate increases in the levels of intracellular cAMP in VSM cells via binding prostanoid/G-protein coupled receptors. Increases in cAMP inhibit myosin light chain phosphorylation, causing relaxation, as well as inhibiting proliferation [137]. Similarly, the endogenous vasodilator nitric oxide (NO), which is released by endothelial cells, also inhibits both myosin light chain phosphorylation (via increases in cGMP) and proliferation of VSM cells [36]. Various NO donors such as glycerine trinitrate and sodium nitroprusside (SNP) have been used in the treatment of vascular disease and endothelial dysfunction (*e.g.,* angina pectoris and hypertension) [138].

7.2. Growth factors

Growth factors such as PDGF, TGF-β and VEGF which regulate smooth muscle proliferation and size, may also be potential targets for the treatment of vascular and lung obstructive diseases [15]. Imatinib, a receptor tyrosine kinase inhibitor which inhibits PDGF signaling, attenuates both VSM cell hyperplasia and hypertrophy in pre-clinical models of vascular disease [139]. However, imatinib was withdrawn from clinical trials for the treatment of advanced PAH, because of serious side effects and increased morbidity [140]. Inhibiting specific aspects of TGF-β signaling may be another growth factor-targeting strategy to treat tissue remodeling in disease. Aberrant TGF-β signaling, important in regulating smooth

muscle function including cell stiffness and proliferation [141, 142], contributes to tissue remodeling in vascular and respiratory diseases [143-145]. The TGF-β superfamily member, activin A, is linked with the progression of PAH, stimulating VSM cell proliferation [141]. Administration of follistatin, an endogenous inhibitor of activin A, attenuates inflammation and remodeling in experimental models of lung injury and disease [146].

7.3. MMPs

Targeting proteases that modify smooth muscle ECM structure and its biomechanical properties are another strategy to treat vascular and obstructive lung disease. Inhibition of pericellular collagenolysis *in situ* may reduce ASM cell hyperplasia in AWR by maintaining the anti-proliferative effects of fibrillar pericellular collagen. Administration of the MMP inhibitory antibiotic doxycycline reduces airway hyper-responsiveness in allergen-challenged mice [147], supporting the concept that interventions with selective MMP inhibitors could be beneficial in the treatment of diseases such as asthma (see Table 1). In preclinical models of vascular disease and injury, doxycycline has been shown to attenuate acute pulmonary thromboembolism (APT)-evoked pulmonary hypertension and right ventricular dysfunction [148].

The effects of targeting ECM proteases in experimental models of lung and vascular disease

Target	Approach	Model (challenge)	Outcome (as compared to wild type or vehicle control treated mice following challenge or injury)
Annexin A2	Genetic (annexin A2 knockout)	Allergen	Reduced airway inflammation [84].
FXa	Pharmacological (Fondaparinux)	Allergen	Reduced AWR. Administered after AWR was established [149].
Lysyl oxidase	Pharmacological (β-aminopropionitrile)	Arterial injury	Reduced perturbations to right ventricular systolic pressure, right ventricular hypertrophy, and vessel muscularization and normalized collagen cross-linking and vessel matrix architecture
MMPs	Pharmacological (doxycycline)	Allergen	Reduced airway hyper-responsiveness [147].
MMPs	Pharmacological (doxycycline)	APT	Reduced hypertension and right ventricular dysfunction [148].
Plasminogen	Pharmacological	Allergen	Reduced eosinophil and lymphocyte numbers, mucus production, and collagen deposition in the lungs [74].

The effects of targeting ECM proteases in experimental models of lung and vascular disease

Target	Approach	Model (challenge)	Outcome (as compared to wild type or vehicle control treated mice following challenge or injury)
	(tranexamic acid) and genetic (plasminogen knockout)		
Plasminogen	Genetic (plasminogen knockout)	Bleomycin	Reduced alveolar macrophage / Increased lung collagen [150].
Plasminogen	Genetic (plasminogen knockout)	Hypoxia	Reduced pulmonary hypertension and pulmonary vascular remodeling [75].
Plasminogen	Genetic (plasminogen knockout)	Hypoxia	No effect [75].
PAI-1	Pharmacological (IMD-1622)	Arterial injury	Reduced arterial neointimal formation, increases in adhesion molecules, fibrinogen accumulation [151]
PAI-1	Genetic (PAI-1 knockout)	Allergen	Reduced AWR including sub-epithelial fibrosis [61, 62].
PAI-1	Genetic (PAI-1 knockout)	Bleomycin	Reduced lung collagen [152].
PAI-1	Pharmacological (Tiplaxtinin)	Allergen	Reduced AWR [153]
PAI-1	Genetic (PAI-1 knockout)	Endotoxin	Reduced neutrophil recruitment to the lungs [154] and fibrin deposition and AWR in the airways [155].
PAI-1	Genetic (PAI-1 knockout)	Arterial injury	Reduced arterial neointimal formation [156].
PAR-1	Pharmacological (ER-112780-06)	Allergen	Reduced AWR [98].
PAR-1	Genetic (PAR-1 knockout)	Bleomycin	Reduced collagen accumulation in lung and pulmnonary inflammation [102].
PAR-1	Pharmacological (Atopaxar)	APT	Reduced neointimal thickening in arterial blood vessels [157]
PAR-1	Pharmacological (F16618)	Balloon angioplasty	Reduced restenosis [158]
tPA	Therapeutic (nebulized tPA)	Allergen	Reduced airway hyper-responsiveness [52]

The effects of targeting ECM proteases in experimental models of lung and vascular disease

Target	Approach	Model (challenge)	Outcome (as compared to wild type or vehicle control treated mice following challenge or injury)
tPA	Genetic (tPA knockout)	Bleomycin	Increased lung collagen/ alveolar hemorrhage [150].
tPA	Genetic (tPA knockout)	Hypoxia	Had no effect on pulmonary hypertension or pulmonary vascular remodeling [75].
uPA	Therapeutic (anti-uPA antibodies)	Endotoxin	Reduced inflammation and edema [159]
uPA	Genetic (uPA knockout)	Bleomycin	Reduced alveolar macrophages [150].
uPA	Genetic (uPA knockout)	Endotoxin	Reduced lung edema, pulmonary neutrophil accumulation, and pro-inflammatory cytokine levels [160].
uPA	Genetic (uPA knockout)	Hypoxia	Reduced pulmonary hypertension and pulmonary vascular remodeling [75].
uPAR	Genetic (uPAR knockout)	Bleomycin	Reduced alveolar macrophages [150].

Table 1. A summary of findings from studies that have targeted ECM proteases and receptors in experimental models of lung and vascular disease. The animal models used were: (i) Acute pulmonary thromboembolism (APT), Which is induced with autologous blood clots; (ii) Allergen, A model of allergic airway inflammation which has characteristics of asthma pathology. In the model, antigen sensitized animals are repeatedly challenged with aerosolized antigen (e.g., ovalbumin, house dust mite extract); (iii) Balloon angioplasty, A vascular intervention procedure which causes restenosis; (iv) Bleomycin, Intranasal administration of bleomycin induces acute lung injury and subsequent pulmonary inflammation and fibrosis. This model also features extensive remodeling in the upper airways involving ASM cell hypertrophy and hyperplasia; (v) Endotoxin, Intranasal administration of endotoxin (i.e., lipopolysaccharide or LPS) also induces acute lung injury and pulmonary inflammation; and (vi) Hypoxia, A model of pulmonary arterial hypertension (PAH).

7.4. Coagulants

Reducing the accumulation of extravascular fibrin in damaged tissue would be expected to reduce tissue remodeling in disease. Anti-coagulants are already used in the treatment of vascular disease to reduce thrombosis by disrupting hemostasis. Whilst clinical asthma trials with nebulized heparin have provided mixed results [161], systemic administration of *fondaparinux*, a selective inhibitor of FXa, attenuates AWR in experimental asthma [149] (table 1). The success of anticoagulant therapy in treating asthma and other respiratory diseases may depend on the anticoagulant, which differ in potency for coagulation-dependent and -independent targets. Selective small molecule FXa inhibitors (*e.g.*, *apixaban*) are used as treatment for venous thromboembolism and stroke prevention in patients with atrial fibrillation [162], but still have anti-coagulant related bleeding risks, which can be potentially fatal.

The coagulants FXa and thrombin may also contribute to tissue remodeling via their activation of PAR-1 on stromal cells including ASM and VSM cells. PAR-1 antagonists reduce AWR in a murine model of chronic allergic airway inflammation [98] and pulmonary inflammation and fibrosis in mice following bleomycin-induced lung injury [102, 103] (table 1). Similarly PAR-1 antagonists reduce restenosis [158] and intimal thickening [157] in the vascular wall following balloon injury *in vivo* (table 1). Orally administered *Vorapaxar*, a PAR-1 inhibitor, is used as anti-platelet therapy for prevention of secondary thrombotic cardiovascular events in patients with a prior myocardial infarction [163]. However, it is because of the ability to suppress thrombin-stimulated platelet aggregation, thus disrupting hemostasis, that PAR-1 inhibitors such as *Vorapaxar* are associated with potentially fatal bleeding complications [164]. For PAR-1 inhibitors to be used as treatment for tissue remodeling disease, they will need to be modified to selectively target the extravascular cell-mediated actions of coagulant proteases without disrupting hemostasis. Taking advantage of integrin co-receptors and adaptors that differentiate PAR-1 responses in platelet and endothelial cells as compared to inflammatory and extravascular structural cells (*e.g.* smooth muscle cells) may be one approach to achieve selectively in regards to PAR-1-drug targeting.

7.5. Fibrinolytic agents

Potential therapies that reduce fibrin by using fibrinolytic agents, have been considered in the treatment of lung disease for some time [165]. Thrombolytic therapy using tPA-mimetics are already used for stroke and myocardial infarction, despite a higher risk of bleeding complications [166]. A potential strategy to treat asthma is to augment airspace fibrinolysis. Tiplaxtinin, a small molecule inhibitor of PAI-1 [153], or inhaled tPA [52], attenuate AWR or reactivity in allergen-challenged mice (table 1). In animal studies of vascular injury and disease, PAI-1 inhibitors [151] or PAI-1 gene deletion [156] reduced neointima formation.

7.6. uPA and annexin A2

Both uPA and annexin A2 may be potential novel drug targets in the treatment of chronic respiratory disease [167]. Both uPA and annexin A2 gene-deletion reduces pulmonary inflammation in various murine models [84, 150, 160], and uPA antibodies reduce inflammation and edema in a mouse model of acute lung injury [159] (table 1). However, further pre-clinical characterization of these inhibitors as therapy for tissue remodeling diseases is required. The roles of uPA and annexin A2 in cancer are well established, and their targeting by either pharmacological or antibody-based therapies have been shown to reduce tumour growth and/or metastasis in a number of pre-clinical cancer models [86-90]. Furthermore, clinical trials for cancer have shown uPA inhibitors are well tolerated in humans and have generated promising results [87].

7.7. Cross-linking enzymes

As the expression of lysyl oxidases are dysregulated in PAH, modulation of lung matrix cross-linking may limit pulmonary vascular remodeling associated with PAH. In support, β-

aminopropionitrile, an inhibitor of lysyl oxidase, attenuates the effect of hypoxia on vascular remodeling in experimental PAH [9] (table 1).

7.8. Actin-cytoskeleton

Cytoskeletal changes in smooth muscle cells occur in association with increases in stiffness (*i.e.*, elastic modulus), size (hypertrophy) and migration [168-170]. A strategy that softens cells by regulating the formation or depolymerisation of the actin-cytoskeleton may be beneficial in the treatment of vascular and lung obstructive diseases. However, there are yet no actin-targeting drugs used clinically [171]. Cytochalasins and latrunculins are cytotoxic as they non-selectively disrupt actin microfilaments. However, there are different populations of actin filaments, spatially organised into distinct cellular compartments with unique functions. Their organization is regulated by associated-actin binding proteins. Therapeutically, actin filament populations need to be targeted specifically, by modulating the binding of specific actin-binding proteins. One approach maybe to target the assembly of FA complexes. Phosphory-lation of FAK leads to disassembly of FA complexes-actin filaments [172]. ECM proteins (*e.g.*, collagen and fibronectin) that engage integrins regulate FAK activity and actin-cytoske-leton organization [173]. FAK regulates actin-cytoskeleton organisation by modulating the association of actin with actin-capping proteins such as gelsolin [174]. Complexes between FAK, gelsolin and the actin-cytoskeleton also regulate gene expression of contractile proteins (*e.g.*, α-smooth muscle actin, calponin and transgelin [SM22]) via the actin-filament-dependent transcriptional co-activator, MRTF-A (myocardin-related transcription factor-A) [174].

8. Conclusion

Understanding the molecular mechanisms by which the ECM regulates smooth muscle cell function in disease remains a key challenge to developing effective therapeutics for managing tissue remodeling diseases such as obstructive lung and vascular diseases. Insight into the biology of the smooth muscle cell in these diseases has increased rapidly in the recent years, leading to the concept that successful strategies for managing tissue remodeling may include restoring smooth muscle phenotype. Such approaches could possibly involve modulating the ECM of the smooth muscle microenvironment, or the factors which are involved in the transmission of the biochemical and biomechanical properties of the ECM. Proteases of the coagulation and plasminogen activation systems are of particular interest as therapeutic targets, as they not only have roles in forming and modifying ECM, but also can directly stimulate changes in smooth muscle cell function and phenotype. The challenge is to target these proteases without disrupting their roles in hemostasis, in order to avoid bleeding complications.

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