Thrombin-mediated cellular events in pulmonary fibrosis associated with systemic sclerosis (scleroderma)

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ABSTRACT

The vascular hypothesis for the pathogenesis of systemic sclerosis was perhaps Professor LeRoy's most important scientific contribution. One early and important consequence of vascular injury is the release of activated thrombin. In this manuscript we present our data and review the current understanding of the role played by thrombin in the process of fibrosis, particularly as it relates to scleroderma lung disease. Thrombin's cellular effects are intimately involved in promoting myofibroblast differentiation, endothelial cell activation, extracellular matrix protein deposition, and the induction of important profibrotic factors. Such studies confirm that thrombin is one of the major mediators in the development and progression of pulmonary fibrosis. Therefore, targeting the major receptor of thrombin, PAR-1, and its downstream signaling molecules may lead to novel therapeutic approaches for the management of scleroderma lung fibrosis. We are indebted to Dr. LeRoy for his many contributions to the field of scleroderma, and for all that he did to stimulate our interest in these studies.

Introduction

Pulmonary fibrosis in systemic sclerosis (SSc, scleroderma) is an irreversible and progressive disease process often leading to death (1-3). Characterized by microvascular and tissue injury and inflammation, it culminates in excessive deposition of extracellular matrix (ECM) proteins, often resulting in severe lung dysfunction (3-6). Cells predominantly responsible for ECM accumulation in the lung are activated fibroblasts or myofibroblasts (7-13). The presence of myofibroblasts in human and animal models of pulmonary fibrosis is now well documented (14-17). Myofibroblasts may arise from differentiation of fibroblasts or other precursor cells, e.g. pericytes, endothelial cells, epithelial cell and fibrocytes (17-20). Myofibroblasts appear to be the principal mesenchymal cells responsible for tissue remodeling, collagen deposition, and the restrictive nature of the lung parenchyma associated with pulmonary fibrosis (8-10,13,16).

The conceptual process of fibrogenesis involves the presence of tissue injury, the release of fibrogenic factors, and the induction of myofibroblasts, culminating in enhanced extracellular matrix deposition (2, 3, 21-23). Several factors capable of inducing the myofibroblast phenotype have been described. Transforming growth factor-β1 (TGF-β1), a factor that also plays a central role in promoting ECM protein synthesis, is perhaps the best studied. Recently, we have reported that the serine protease, thrombin, also mediates the differentiation of lung fibroblasts to a myofibroblast phenotype, apparently at an even earlier stage than TGF-β1 (27, 28).

In recent years, increasing evidence has accumulated to implicate involvement of the coagulation system in various fibrotic diseases, including pulmonary fibrosis (29-36). Activation of coagulation proteases, e.g. thrombin, is one of the earliest events following tissue injury (29, 33, 36). Activation of the coagulation system and generation of thrombin following injury modulates tissue repair responses by altering vascular permeability, stimulating fibroblast and neutrophil migration, and promoting the adhesion and spreading of endothelial cells, epithelial cells and fibroblasts (33-37). Therefore, during lung injury thrombin activates various cell types and induces the secretion of several profibrotic and angiogenic factors (37-42). Activation of these cells by thrombin is a likely mechanism for the development and progression of pulmonary fibrosis in general, and lung

fibrosis associated with scleroderma in particular.

Role of thrombin in pulmonary fibrosis

There is compelling evidence that the serine protease and G protein coupled receptor ligand, thrombin, is an important mediator of idiopathic pulmonary fibrosis (IPF), interstitial lung fibrosis associated with SSc, chronic asthma, and animal models employing bleomycin to induce lung fibrosis (30-40, 44-48). We, as well as others, have demonstrated elevated levels of thrombin in bronchoalveolar lavage (BAL) fluid from scleroderma patients with lung fibrosis (29-31), as well as for other fibrosing lung diseases (36). We found that BAL fluids from normal subjects contain a low level of thrombin activity (48.6 ± 8.7 U/mg of protein), while BAL fluids from SSc patients contain significantly higher thrombin activity (699.9±201) (29).

Thrombin is a potent mitogen for lung fibroblasts (27-31). Previously, we reported that the mitogenic effect of thrombin on human lung fibroblasts is mediated mainly via PDGF-α receptor up-regulation and enhancement of PDGF-AA ligand expression (29). Recently we demonstrated that thrombin-induced DNA synthesis in human lung fibroblasts is mediated by PKCα and RhoA (48). Thrombin also induces the expression of profibrogenic factors, e.g. transforming growth factor-β (TGF-β1), in smooth muscle cells and epithelial cells (39,47). Moreover, thrombin is a potent stimulator of connective tissue growth factor (CTGF) in fibroblasts (36,49-51). Both of these growth factors are known to participate in various fibrotic diseases, including SSc pulmonary fibrosis (43, 49, 50-55). Levels of each are elevated in BAL fluids and sera from scleroderma patients (43). CTGF mediates some of the profibrotic functions of TGF-β and has been shown to act synergistically with TGF-β to promote chronic fibrosis (54, 55). The presence of the active form of thrombin, together with PAR-1 and CTGF, was recently demonstrated in bleomycin-induced pulmonary fibrosis (49). It has also been demonstrated that CTGF mediates matrix production in lung fibroblasts, which seems to be central to the development of the fibrogenic response, and that inhibition of CTGF activity may be an effective treatment for pulmonary fibrosis (49). Overexpression of CTGF in various fibrotic tissues, including lung, has been observed, with myofibroblasts being the cells mainly responsible for CTGF production (43, 54, 55). High levels of CTGF in lung tissue have been shown to correlate with high collagen synthesis in scleroderma patients (43).

Thrombin has been demonstrated to enhance extracellular matrix proteins, e.g. fibronectin, by epithelial cells and fibroblasts, and procollagen by smooth muscle cells (37, 38, 40) and endothelial cells (41). Studies from our laboratory have shown that thrombin is a potent inducer of tenascin C in human lung fibroblasts (35). We have also shown that thrombin induces interleukin-8 (IL-8) in lung fibroblasts (34). Levels of IL-8 are elevated in BAL fluids of scleroderma patients (56). Thus, thrombin is a potent inducer of profibrotic factors and ECM proteins in various cells within the lung micro-environment. Each of these factors is believed to play an important role in the development and progression of SSc lung fibrosis (29, 34-36, 51-58).

Thrombin induces a myofibroblast phenotype resistant to apoptosis

The appearance of myofibroblasts in areas of active fibrosis, together with the results of *in vitro* studies, strongly suggests that myofibroblasts are key contributors to the increased extracellular matrix synthesis characteristic of SSc and other interstitial lung fibroses (8-10, 35, 59). Myofibroblasts can be cultured from BAL fluids of scleroderma patients with interstitial fibrosis (57). Previously, we reported that such cells express more collagen I, collagen III and fibronectin than do normal lung fibroblasts, and that they have an enhanced proliferative response upon ex-

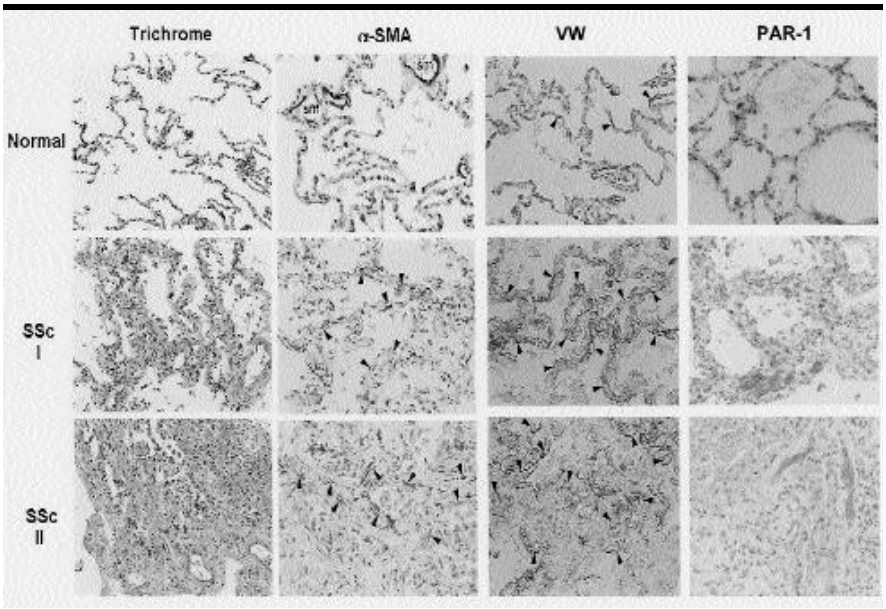

Fig. 1. **α-SMA, VWand PAR-1 expression is significantly increased in pulmonary fibrosis associated with scleroderma.** Masson's trichrome (blue staining) immunostaining for smooth muscle-α actin (α-SMA) expression, von Willebrand factor (VW) and proteolytically activated receptor-1 (PAR-1) in normal and SSc lung tissues. Sections of lung were immunostained with mouse monoclonal antibody for α-SMA, von Willebrand factor and PAR-1 (ATAP2 raised against amino acids 42-55 of human thrombin receptor, PAR-1). α-SMA, VW and PAR-1 are visualized as brown color using immunoperoxidase method with diaminobenzidine (DAB) as a substrate and counterstained with hematoxylin. **Top panel**: normal lung tissue; **middle panel**: SSc I - represents lung tissue in early stage of lung involvement; **bottom panel**: SSc II - represents lung tissue with late stage of lung fibrosis. Sm, indicates sm-α actin in blood vessel endothelial cells. **Black arrows** indicate sm-α actin expression in myofibroblasts, von Willebrand factor in endothelial cells. PAR-1 is expressed both in myofibroblasts and endothelial cells.

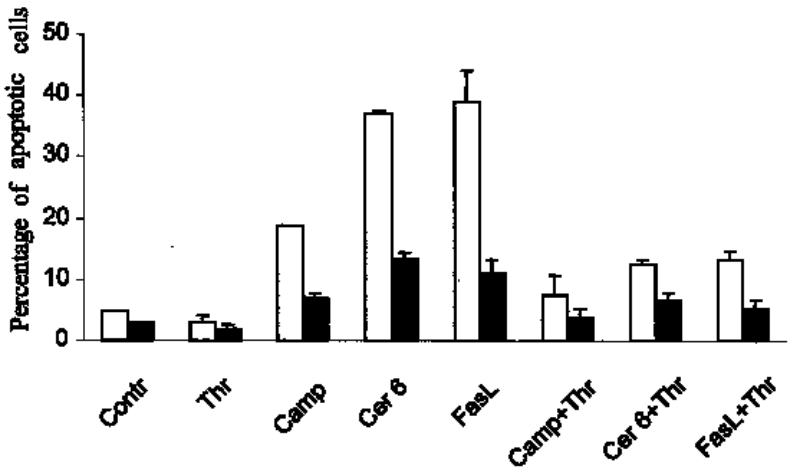

Fig. 2. Thrombin makes normal and scleroderma (SSc) lung fibroblasts resistant to apoptosis. Normal and SSc lung fibroblasts were grown to 80% confluency and then stimulated with Camp (camptothecin 16 μM), Cer (ceramide 6, 20 μM) and FasL(20 ng/ml) in the presence or absence of Thr, thrombin (0.5 U/ml) in serum-free medium for 24 hours. **Contr**: control cells were incubated in serum-free medium only. **White bars** represent normal lung fibroblasts and **black bars** represent SSc lung fibroblasts. For the flow cytometry analysis, single cell suspensions were prepared by trypsinization. Cells were then fixed in 70% ethanol and stained with propidium iodine (120 mg/ml). Cell fluorescence was measured with a FACS scan flow cytometer. A negative control gate was set using cells incubated in serum-free medium only. A minimum of 10,000 events were collected per sample. Measurement of fluorescence was performed at > 620 nm. Apoptotic cells are expressed as a percentage of the total cells in the population. Thrombin induces resistance to apoptosis induced by camptothecin, ceramide and FasL.

posure to TGF-β1 and PDGF compared with normal lung fibroblasts (58). Myofibroblasts, the main source of the profibrogenic factor CTGF (55), are present in lung tissue of scleroderma patients with active lung fibrosis (10, 27, 28) (see Fig. 1).

Thrombin activates and differentiates normal lung fibroblasts to a myofibroblast phenotype via PAR-1 and via PKCε signaling (27). When normal lung fibroblasts are stimulated with thrombin, smooth muscle-α actin (SM-α) interacts with PKCε and serves as a substrate for this PKC isoform. The SM-α-PKCε complex exists *de novo* in untreated fibroblasts derived from scleroderma lung, presumably due to activation *in vivo*. Myofibroblasts regulating fibrotic processes, including SSc, are often resistant to apoptosis, and their persistence in the injured lung is postulated to contribute to the pathogenesis of lung fibrosis (60, 61). Apoptosis, or programmed cell death, is a fundamental process in cellular homeostasis and forms part of normal development and tissue turnover (62-65). Repair after lung injury requires the elimination of proliferating mesenchymal and inflammatory cells from the alveolar airspace or alveolar wall (64). Malfunction of apoptosis, or failure by apoptosis to remove unwanted cells, may prolong inflammation and/or the proliferation of fibroblasts, leading to fibrosis.

Apoptosis triggered by activation of the Tumor Necrosis Factor Receptor (TNFR) superfamily has been shown to be associated with the development of pulmonary fibrosis (66-68). Membrane receptor Fas (CD95), a member of the TNFR family, and its natural ligand, FasL, are expressed in various cells and tissues, including lung (66, 67). FasL exists as a membrane-bound form and a soluble form (sFasL), each of which can activate Fas (64-66). In recent years, apoptosis mediated by Fas-Fas ligand in the lung has been implicated as an important pathway in inflammation and pulmonary fibrosis (66-68). Resistance to Fas-induced apoptosis is seen in pathological conditions, such as autoimmunity and hematological malignancy (68). It has been demonstrated that dermal scleroderma fibroblasts are specifically resistant to apoptosis induced by Fas receptor stimulation, while normal dermal fibroblasts are sensitive to this apoptotic stimulant (58, 59), for which a role of TGF-β has been postulated (60-62).

Our studies indicate that lung myofibroblasts isolated from SSc patients are resistant to several apoptotic stimuli, including FasL-induced apoptosis (48) (see Fig. 2). Furthermore, we have demonstrated that normal lung fibroblasts stimulated with thrombin became resistant to FasL-induced apoptosis and exhibit a phenotype similar to that observed in scleroderma lung, supporting a role for thrombin in cell survival (27, 28). Fibronectin and tenascin-C, each induced in lung fibroblasts by thrombin (30, 35), have been shown to protect fibroblasts from apoptosis (68), preserving the myofibroblast phenotype. Thrombin has been also shown to induce cell survival in other cell systems (70, 71).

Thrombin signaling via protease-activated receptor, PAR-1, and its downstream targets

Protease-activated receptors (PARs) are G-protein-coupled receptors that convert an extracellular proteolytic cleavage event into a transmembrane signal (72-78). Unlike most G-protein-coupled receptors, PARs carry their own ligands that are unmasked by receptor cleavage (72, 73). PAR-1 is widely distributed in many different cell types, including fibroblasts, and appears to be the predominant human thrombin receptor mediating the cellular action of thrombin (38) (Fig. 1). Recent studies have shown levels of PAR-1 to be elevated in kidney and liver fibrosis (72, 74-76). PAR-1 is also highly elevated after bleomycin-induced pulmonary injury, where it is predominantly localized to macrophages and fibroblast-like interstitial cells in fibroproliferative foci (49). Recently, we demonstrated that PAR-1 is markedly upregulated in the lungs of scleroderma patients, where it coincides with increased numbers of myofibroblasts and endothelial cells (48) (Fig. 1).

Most PAR-1 signaling is mediated through heterotrimeric G proteins, with the exact intracellular signaling cascade being cell-type specific (72). Thrombin activates PAR-1 by proteolytic cleavage at the R41/S42 site of the

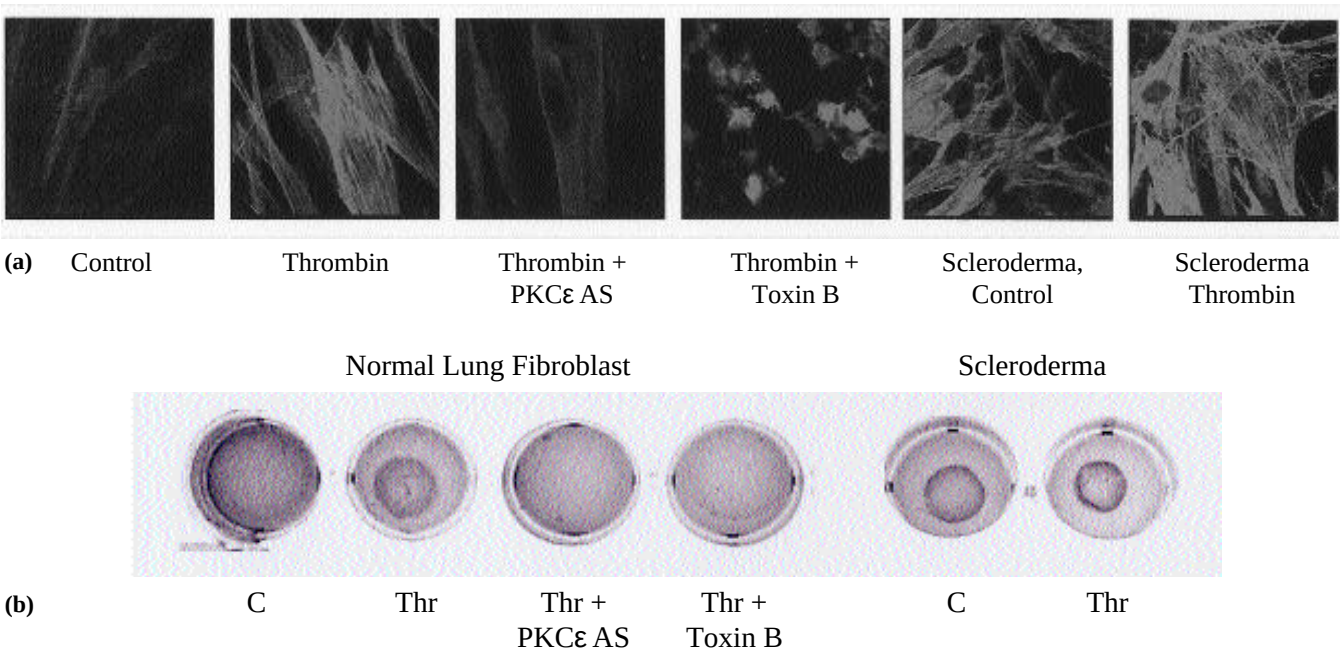

Fig. 3. Depletion of PKCε or inhibition of RhoAabolishes thrombin-induced smooth muscle-α actin organization and collagen gel contraction in normal lung fibroblasts. SM-α actin expression and organization in normal (Nml) and scleroderma (SSc) lung fibroblasts analyzed by confocal microscopy after 24 h of thrombin stimulation (0.5 U/ml). Measurements of gel diameter in collagen gel contraction assay were taken after 2 h incubation with thrombin (0.5 U/ml). Normal lung fibroblasts were treated with antisense (AS) oligonucleotides for PKCε (2 μM) or with Rho inhibitor, toxin B (TB) (50 pg/ml). Note that PKCε depletion abolished thrombin-induced sm-α actin expression, organization and collagen gel contraction in lung fibroblasts. Toxin B inhibited sm-α actin organization and collagen gel contraction in lung fibroblasts stimulated with thrombin but did not abolish sm-α actin expression. The thrombin-induced myofibroblast phenotype was observed in untreated SSc lung fibroblasts. (Portions of this material have been published previously and are used with permission, see References 27 and 28).

N-terminal extracellular domain, converting the inactive receptor to an active form capable of interacting with multiple G proteins, e.g. Gi, Gq, G12/13, in the same cell (75,76,78). Gq-dependent signaling activates phospholipase C, which leads to phosphoinositide hydrolysis and results in Ca^{++} mobilization and activation of protein kinase C (PKC). PKC is required for DNAsynthesis as well as smooth muscle cell growth (72), both necessary events in thrombin-mediated cell proliferation (72,75,76). Gβγ subunits of Gi-protein activate phoshoinositide-3-kinase (PI-3K) followed by Akt phosphorylation, which protects cells from apoptosis (64, 68, 69). The α subunit of G12 and G13 binds Rho guanidine-nucleotide exchange factors, activating small G-protein RhoA and mediating cytoskeletal reorganization (79-81). Our recent studies demonstrate that the PKCα/MAPK and PI3K/p70S6 kinase pathways represent key signaling routes in thrombin-induced lung fibroblast (82).

PKC and Rho pathways in thrombin-activated myofibroblasts

In previous studies we demonstrated that thrombin induces IL-8 and tenascin-C via activation of PAR-1 and downstream through activation of PKCγ and PKCε, respectively (34, 35). Thrombin-induced differentiation of normal lung fibroblasts to a myofibroblast phenotype is mediated by PAR-1 (27) and by multiple downstream signaling pathways such as PKC and Rho (27,28) (Fig.3). Depletion of PKCε, inhibition of PKCε or inhibition of Rho activation abolishes thrombin-induced SM-α expression/organization and collagen gel contraction by lung fibroblasts (27,28), suggesting a role for each in myofibroblast differentiation.

The molecular link between Rho and actin stress fiber formation has recently been identified (79). The downstream target of Rho, Rho-kinase, directly phosphorylates the myosin light chain, promoting interaction of myosin filaments with actin filaments, followed by stress fiber formation and increased contractility (83). Thrombin has been shown to promote actin reorganization in endothelial and astrocytoma cells via Rho-dependent activation of myosin light chain, but without PKC involvement (79,83). Our results indicate that thrombin rapidly activates Rho and significantly increases Rho-[^{35}S]GTPgS binding in lung fibroblasts (28). Thrombin strongly stimulates Rho activity *in vivo* and initiates PKCε-RhoAcomplex formation (28). The Rho inhibitor, toxin B, which inactivates Rho by ADP ribosylation, inhibits thrombin-induced SM-α expression/organization and collagen gel contraction; toxin B also inhibits PKCε/SM-α actin complex formation and PKCε/RhoA co-immunoprecipitation by lung fibroblasts (28). Moreover, we have demonstrated that PKCε/RhoA complex formation is an early event in thrombin's activation of lung fibroblasts (28).

Using a yeast two-hybrid system, direct interactions between the yeast homologues of Rho protein, Rho1p, and the homologues of mammalian PKC, Pkc-

1p, have been shown (83). Recent studies also provide evidence that mammalian PKC isozymes and Rho GTPases co-immunoprecipitate and participate in direct protein-protein interactions (84). The association of PKCα with Rho has been demonstrated in endothelial cells, suggesting a critical role in Rho activation (85, 86). However, we have observed that Rho protein is not necessary for thrombin-induced PKCε activation and translocation to the membrane, nor does depletion of PKCε affect Rho activation and thrombin-induced GTPgS binding (28). On the other hand, Rho inhibition prevented co-immunoprecipitation of PKCε with SM-α actin, suggesting that the association of thrombin-activated Rho with PKC is essential for the regulation of SM-α actin organization in lung fibroblasts (28).

We recently found that overexpression of both constitutively active PKCε and constitutively active RhoA induces highly organized SM-α actin and the contraction of collagen gels; individually, neither was capable of these functions (28). Studies have shown that Rho alone can increase the activity of SM-α actin promoter and modulate SM-α actin expression in other cell types, such as vascular smooth muscle cells (81). However, our data suggest that PKCε, in association with RhoA, recruits SM-α actin and possibly some other protein(s) to promote the intracellular events responsible for SM-α actin expression and organization. Thus, PKCε- and Rho-mediated signaling pathways are essential for thrombin-induced differentiation of normal lung fibroblasts to the scleroderma myofibroblast phenotype. Based on our recent observations and data from the literature, we have proposed a hypothetical mechanism for the development of myofibroblast differentiation leading to pulmonary fibrosis (see Figure 4).

Thrombin and microvascular alterations in pulmonary fibrosis

Microvascular changes, such as the enhanced proliferation of endothelial cells and neovascularization, are observed in lung fibrosis (87-98). Few studies, however, have addressed the importance of vascular remodeling in the lung during the course and development of pulmonary fibrosis. We have observed the proliferation of microvascular endothelial cells and the generation of functional capillaries in scleroderma lung fibrosis (Fig. 1). Early vascular dysfunction in scleroderma lung may result from the hyperproliferation of endothelial cells and possibly from the increased numbers of alveolar capillaries (hypervascularity). These microvascular alterations are seen most prominently during early phases of pulmonary fibrosis (Fig. 1) Similarly, enhanced proliferation of endothelial cells and neovascularization mediated by IL-8 and IL-10 have been observed in IPF (89), as well as in bleomycin-induced pulmonary fibrosis in mice (90).

Thrombin has been shown *in vitro* and *in vivo* to promote endothelial cell activation followed by angiogenesis, and this also is mediated by PAR-1 and via PKC (73). Interestingly, abrogation of thrombin-induced increases in pulmonary microvascular permeability has been shown in PAR-1 knockout mice (98). The authors suggest that PAR-1 is critical in mediating the permeability-increasing and vasoconstrictor effects of thrombin in pulmonary microvessels (98). Moreover, thrombin-stimulated lung fibroblasts secrete several proan-

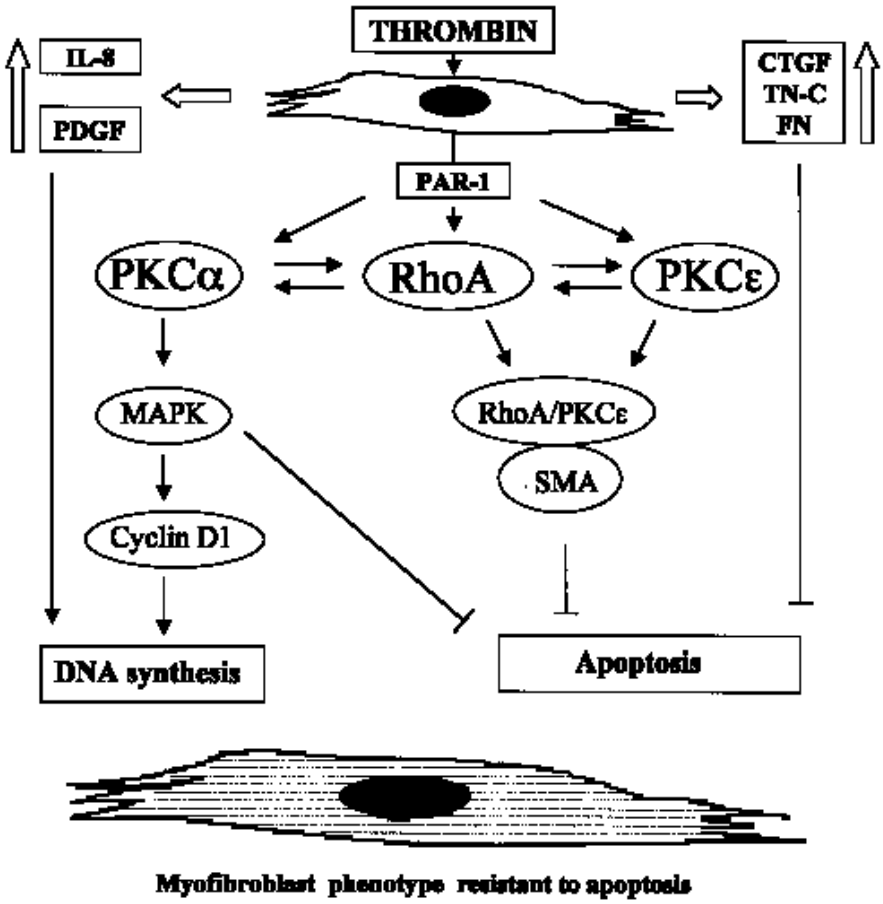

Fig. 4. Proposed schema for thrombin-activated lung fibroblast and differentiation to a myofibroblast phenotype resistant to apoptosis. Thrombin, via its proteolytically activated receptor PAR-1, activates PKCα, PKCε and RhoA. Activation of PKCε and RhoAresults in PKCε/RhoAimmunocomplex formation followed by the formation of a complex with SM-α actin (SMA). Ternary complex PKCe/RhoA/SMAresults in significantly increased SM-α actin expression and organization, causing differentiation of normal lung fibroblasts to a myofibroblast phenotype. Activated PKCε and RhoA inhibit FasL-induced apoptosis, resulting in transformation of the lung fibroblast to a phenotype that is resistant to apoptosis. Additionally, activated PKCα mediates thrombin-induced DNAsynthesis in lung fibroblasts and participates in the differentiation of these cells. Factors induced by thrombin in lung fibroblasts, e.g. PDGF, mediate DNAsynthesis, while tenascin C (TN), fibronectin (FN) and connective tissue growth factor (CTGF) inhibit apoptosis in lung fibroblasts and are involved in the induction of resistance to apoptosis.

giogenic factors, e.g. metalloproteinases (MMP's), which increase endothelial cell invasion, and VEGF, VCAM-1, IL-8, PDGF, PAI-1 and tenascin-C (91, 93-96, 99). As noted above, thrombin induces CTGF in fibroblasts, promoting endothelial cell proliferation and neovascularization *in vivo* (51, 55). Thus, thrombin induces an angiogenic phenotype in microvascular endothelial cells *in vitro* and promotes angiogenesis *in vivo*. The angiogenic action of thrombin via PAR-1 has also been demonstrated in tumor progression and metastases, including in lung (98). The presence of thrombin in many disease conditions in which neovascularization is activated, such as inflammation, atherosclerosis and cancer, as well as in lung fibrosis, suggest a pivotal role for thrombin in the progression of such diverse pathological conditions (37).

Role of thrombin in cross talk among activated lung fibroblasts, epithelial cells and endothelial cells during the development of pulmonary fibrosis

During the development of lung fibrosis, activated fibroblasts interact with other cell types such as immune cells (macrophages, monocytes, T cells, mast cells and eosinophils) and non-immune cells (epithelial and endothelial cells) (8-10, 95, 100, 101). There is now considerable evidence that myofibroblasts are capable of modulating the properties of other cells through the paracrine activity of soluble product(s) (49,50, 95). Factors secreted by endothelial cells are mitogenic for various mesenchymal cells and stimulate collagen production (53-55). Factors secreted by both cell types promote the migration of these cells to various areas of tissue injury in the lung (100,101). Because thrombin activates lung fibroblasts and increases the secretion of proangiogenic factors from these cells, as well as from endothelial cells, the interaction of these two cell types in the presence of thrombin may be crucial in the development of lung fibrosis. Similarly, factors secreted by epithelial cells are mitogenic for various mesenchymal cells (fibroblasts and smooth muscle cells) and stimulate collagen production (10,95). Epithelial-mesenchymal transformation (EMT), besides its role in embryonic development, tumorigenesis and organ remodeling during fibrogenesis (101), has been shown to participate in the progression of lung fibrosis. Inflammatory factors known to participate in epithelial cell and fibroblast differentiation, such as TGF-β1, IL-8, IL-11 and fibronectin, are induced by thrombin in several different cell types (27, 28, 37, 100, 101). Because all of these cell types utilize similar signaling pathways when activated by thrombin, targeting PAR-1 and/or its downstream signaling molecules may prove to be a powerful approach to the treatment of pulmonary fibrosis.

Thrombin-induced signaling as a potential therapeutic target in lung fibrosis

In an animal model of pulmonary fibrosis, a direct thrombin inhibitor, UK-156406, attenuates lung collagen accumulation by lowering the profibrotic effects of thrombin and suppressing CTGF synthesis (36,49). This peptide inhibits thrombin's proteolytic activity by binding in its catalytic triad (49). Other direct thrombin inhibitors, e.g. lepirudin, bivalirudin, argatroban and melagatran, also interact with thrombin by blocking its catalytic activity, and are currently approved for clinical use in cardiovascular disease (102). Another even more attractive way of targeting thrombin's action is by the inhibition of the thrombin receptor.

The collection of thrombin receptor antagonist(s) currently available falls into several categories: (i) peptide antagonists; (ii) peptidomimetics; (iii) non-peptide thrombin receptor inhibitors; and (iv) statins (103-106). Several peptides with structural similarities to the tethered ligand have been shown to prevent thrombin- and tethered ligand-induced cellular effects (103), but *in vivo* studies are still very limited. Statins, 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase inhibitors, suppress tissue factor, and thus thrombin generation. It has been suggested that statins inhibit PAR-1 expression and desensitize cells to thrombin stimulation (104). Several investigators have demonstrated that statins downregulate the prenylation of the proteins involved in signal transduction, including PKC and Rho (104-106). Selective inhibition of the profibrotic effects of thrombin or PAR-1 by direct thrombin inhibitors or PAR-1 antagonists at the cellular level may avoid potential complications, e.g. thrombocytopenia or thrombosis, associated with the inhibition of thrombin and other coagulation proteases. We believe that targeting thrombin and/or PAR-1 signaling may represent an attractive therapeutic approach for SSc-associated lung fibrosis, as well as other forms of pulmonary fibrosis.

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