Role of coagulation and fibrinolysis in lung and renal fibrosis

C. Ruppert, P. Markart, M. Wygrecka, K. T. Preissner, A. Günther
University of Giessen Lung Center, Germany

Keywords
Coagulation, fibrinolysis, fibrosis

Summary
Elevated procoagulant and suppressed fibrinolytic activities are regularly encountered in different forms of clinical and experimental fibrosis of the lungs and the kidneys. Although primarily serving to provide a provisional matrix of repair largely consisting of fibrin and fibronectin, the involved procoagulant serine proteases and protease inhibitors may also exert distinct cellular downstream signaling events modifying the fibrotic response. In this review, evidence for an impaired regulation of coagulation and fibrinolytic factors in clinical and experimental lung and renal fibrosis is provided and the role of PAR (protease activated receptor) induced profibrotic and antifibrotic cellular events is worked out. In view of experiments obtained in animal models of lung and renal fibrosis, the potential therapeutic usefulness of anticoagulant or profibrinolytic strategies is discussed.

Coagulation and fibrinolysis in interstitial lung disease

In the lungs of patients with interstitial lung diseases (ILD) such as idiopathic pulmonary fibrosis (IPF) (1, 2), increased procoagulant and decreased fibrinolytic activities are commonly observed. This is linked to the severity of disease (3) and results in increased alveolar fibrin deposition due to a local haemostatic imbalance already early in the course of disease (4, 5). Similar changes of the alveolar haemostatic balance have been reported in animal models of acute lung injury and pulmonary fibrosis including that of bleomycin-induced lung injury (6, 7).

In both, experimental and clinical ILD, intraalveolar activation of the coagulation cascade is mainly attributable to the increased expression of tissue factor (TF) and factor (F)VII (1–3). Alveolar epithelial type II cells and alveolar macrophages have been identified as major source of such procoagulant activity (1, 8, 9). The TF/FVIIa initiated activation of FX (10) and activation of thrombin forward off-cleavage of fibrinopeptides from fibrinogen and conversion into fibrin. In clinical and experimental ILD, such increase of procoagulants is also amplified by unchanged TF pathway inhibitor (TFPI) (11) and suppressed activated protein C (APC) levels (12, 13). It was reported that epithelial cells of the lung can express and synthesize fibrinogen (14). Thus, in principle, the lung can regulate generation of procoagulatory factors and deposition of fibrin in an autchthonal manner and independent of the status of barrier function and leakage of plasma proteins.

Parallel to the upregulation of prothrombotic factors, a far-reaching suppression of fibrinolytic activity is encountered in fibrotic lung diseases. This is largely attributable to a strongly increased expression of plasminogen activator inhibitor (PAI)-1 in face of suppression of urokinase-like plasminogen activator (u-PA) under these conditions (1–3, 6, 7).

Local haemostatic imbalance in fibrotic renal diseases

Deposition of fibrin in the periturbar capillaries and the interstitial spaces is also a prominent feature in patients with fibrotic disorders of the kidney (15–21), such as ischaemic nephropathy, obstructive nephropathy, chronic renal allograft or antiphospholipid syndrome nephropathy and in different forms of glomerulonephritis.

In some experimental studies, such as in a model of hydrenephrosis (20), it was shown that TF expression is highly increased in endothelial, glomerular capular, tubular epithelial and infiltrating interstitial cells...
and thus represents as major source of a locally increased procoagulant activity resulting in increased fibrin deposition.

As in the lung, also the fibrinolytic system has been shown to be largely down-regulated in a variety of chronic renal diseases resulting in organ fibrosis. In detail,

- suppression of tissue plasminogen activator (t-PA) or u-PA and
- upregulation of PAI-1 in renal tissue

was detected in diabetic nephropathy (22, 23), several forms of glomerulonephritis (focal necrotizing, crescentic, focal segmental or membranous) (24–29), chronic allograft nephropathy (30–32), thrombotic microangiopathy (33, 34), arteriopathy (35, 36). The role of PAI-1 in chronic kidney disease has been recently reviewed (37).

Pathways contributing to fibrosis

It was previously anticipated that the deposition of fibrin per se may represent an important mechanism in the propagation of organ fibrosis. This was primarily based on the observation that the extent of organ fibrosis was somehow correlated with the severity of procoagulant and antifibrinolytic changes, both in lung fibrosis as well as in fibrotic renal diseases (3, 15). With regard to the lung, it had also been shown that peptides resulting from degradation of fibrinogen impair the barrier function of the distal lung unit and that alveolar formation of fibrin results in an acute induction of severe gas exchange abnormalities due to incoporation of all hydrophobic surfactant compounds into the growing fibrin lattice (38, 39). Moreover, fibrin is thought to promote the fibrotic response by providing a provisional matrix capable of fibroblast proliferation and activation. Provisional matrix components such as fibrin and fibronectin have also been recently disclosed to induce epithelial to mesenchymal transition (EMT) in vitro, a pathophysiological important process in lung and renal fibrosis (40). Based on these results it was speculated that collapsed, fibrin-glued alveoli may represent the nidi of fibrotic processes in the lung.

However, the observation that lung fibrosis may nevertheless develop in fibrinogen knock out mice in response to bleomycin challenge (41, 42), a standard model of lung fibrosis, has raised some doubts in view of the role of fibrin formation itself and has set the stage for the identification of several other, potentially important signaling mechanisms that could additionally contribute to development of organ fibrosis (Fig. 1). The first candidate molecule mentioned in this context is the protease activated receptor (PAR)-dependent signaling pathway. PAR-1–4 are a group of seven transmembrane G-protein-coupled receptors occurring on the surface of platelets, endothelial, interstitial and epithelial cells (43, 44). Upon cleavage of an extracellular, aminoterminal activation domain, the new N-terminus serves as an intramolecular tethered ligand and induces signaling via G-protein-coupled mechanisms. The known four PAR differ in protease sensitivity and specificity (45). Of note,

- thrombin activates PAR-1, -3, and -4,
- FXa mostly PAR-1 and -2,
- TF/FVIIa PAR-2 (43–45).

Apart from the pleiotropic effects in the vasculature (regulation of vessel tone, platelet degranulation, induction of endothelial leakage and proliferation, smooth muscle cell proliferation and matrix production) and immune competent cells (activation of monocytes, T-lymphocytes and mast cells with release of proinflammatory cytokines), PAR-1 signaling not only induces release of platelet-derived growth factor, connective tissue growth factor and transforming growth factor-β, but also fibroblast activation, proliferation, myofibroblast transformation and induction and synthesis of collagen (42–45). Thus, PAR mediated signaling could largely influence profibrotic events in lungs and kidneys.

Still more complicating is the observation, that thrombin/thrombomodulin-dependent induction of activated protein C (APC), at least on the endothelium, would limit coagulation, inflammation and apoptosis (46). These cytoprotective events are dependent on binding of APC at least to PAR-1 and the endothelial APC receptor (EPCR) (47, 48), indicating that PAR-1 may

Fig. 1 Profibrotic versus antifibrotic signaling pathways induced by different serine proteases and serpins

Hämostaseologie 1–2/2008
31
Coagulation and fibrinolysis in fibrosis

For personal or educational use only. No other uses without permission. All rights reserved.

Downloaded from www.haemostaseologie-online.com on 2017-06-17 | IP: 54.191.40.80
have in part diverging cellular downstream effects pending on the nature of the ligand.

Moreover, the fibrinolytic system may exert profound influence on tissue fibrosis and matrix remodeling independent of the process of fibrin(ogen) cleavage. In detail, it had been shown that u-PA and especially plasmin are capable of directly or indirectly activating different matrix metalloproteases (MMP) such as MMP1 and MMP3 (49, 50). The u-PA/uPA-receptor system also plays a significant role in pericellular lysis and thus cell migration (51). Finally, u-PA, the predominant plasminogen activator in lung and kidney, directly activates hepatocyte growth factor (HGF) (52, 53), a cytokine with distinct antifibrotic and antiapoptotic properties on epithelial cells. In detail, activated HGF not only blocks transforming growth factor-β-dependent signaling pathways and connective growth factor activation in epithelial cells on the level of signaling proteins Smad 2,3 via upregulation of the Smad transcriptional co-repressor SnoN (54–56), it is also under discussion to represent an important anti-apoptotic factor of epithelial cells being released by the surrounding mesenchymal cells (57–60). At least in some clinical forms of lung fibrosis such as IPF, the release and the activation of HGF was described as markedly reduced in isolated lung fibroblasts (57, 61).

Altogether, there is considerable evidence that the modulation of fibrin deposition per se may play an inferior role as compared to cellular downstream signaling events in the propagation of organ fibrosis by procoagulant or antifibrinolytic factors.

### In vivo evidence and therapeutic relevance

The respective role of single coagulant or fibrinolytic factors has been assessed in different animal models of lung and renal fibrosis (Tab. 1). With regard to lung fibrosis, all data suggest that in bleomycin-induced lung injury and fibrosis, which is the only model still studied, almost any therapeutic modulation of the haemostatic balance in a sense of increased fibrinolytic or suppressed procoagulant activity is followed by an attenuation of the extent of fibrosis (62–67, 69). As the course of bleomycin-induced lung fibrosis remained largely unchanged in fibrinogen, t-PA and uPAR knock out mice (41, 42, 63), it had been suggested that fibrin per se does not play the dominant role. Rather, u-PA/plasmin- or TF/ FVIIa, FX- or thrombin-elicited downstream signaling pathways may largely determine the magnitude of the fibrotic response. This was reinforced by the observation that gene transfer or protein application of HGF as well as the study of PAR-1 knock-out mice was followed by a far-reaching protection from fibrotic events in the bleomycin model.

Although fairly reproducible and thereby mimicking the disturbed alveolar coagulatory and fibrinolytic balance observed in ILD, it is to mentioned that the bleomycin model of lung fibrosis does not represent an optimal model for some of the most aggressive forms of ILD such as IPF. In more depth: The bleomycin model is largely an inflammatory driven lung fibrosis model. However, IPF is nowadays considered to de-

### Tab. 1 The role of coagulant and fibrinolytic factors in experimental lung and renal fibrosis

<table>
<thead>
<tr>
<th>animal studies in</th>
<th>transgenic or KO mouse model</th>
<th>major observation concerning fibrosis</th>
<th>references</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>lung fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 /-; u-PA overexpression</td>
<td>bleomycin</td>
<td>attenuation</td>
<td>62, 64</td>
</tr>
<tr>
<td>PAI-1 overexpression, u-PA /-, plasminogen /-</td>
<td></td>
<td>augmentation</td>
<td>62, 63</td>
</tr>
<tr>
<td>t-PA /-; uPAR /-</td>
<td></td>
<td>no significant change</td>
<td>63</td>
</tr>
<tr>
<td>fibrinogen /-</td>
<td></td>
<td>attenuation</td>
<td>41, 42</td>
</tr>
<tr>
<td>aPC administration, u-PA administration (anrosal)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HGF protein application or gene transfer</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>unfractioned heparin administration (inhautive)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>thrombin antagonist UK 154606 (i.p.)</td>
<td></td>
<td></td>
<td>66</td>
</tr>
<tr>
<td>PAR-1 /-</td>
<td></td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>renal fibrosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PAI-1 /-</td>
<td>TGF-β transgenic mice crescentic anti-GBM nephritis obstructive nephropathy</td>
<td>attenuation</td>
<td>74–78</td>
</tr>
<tr>
<td>PAI-1 overexpression</td>
<td>crescentic anti-GBM nephritis obstructive nephropathy</td>
<td>augmentation</td>
<td>74, 79</td>
</tr>
<tr>
<td>mutant PAI-1 application (binds vitronectin, but not PA)</td>
<td>anti-Thy-1 nephritis</td>
<td>attenuation</td>
<td>80</td>
</tr>
<tr>
<td>plasminogen /-</td>
<td>obstructive nephropathy crescentic anti-GBM nephritis</td>
<td>augmentation</td>
<td>88, 89</td>
</tr>
<tr>
<td>t-PA /-</td>
<td></td>
<td>attenuation</td>
<td>81</td>
</tr>
<tr>
<td>application of rec. t-PA</td>
<td>anti-Thy-1 nephritis crescentic anti-GBM nephritis</td>
<td>attenuation</td>
<td>83, 84</td>
</tr>
<tr>
<td>u-PA /-</td>
<td>obstructive nephropathy crescentic anti-GBM nephritis</td>
<td>no major change</td>
<td>74, 85</td>
</tr>
<tr>
<td>uPAR /-</td>
<td>obstructive nephropathy crescentic anti-GBM nephritis</td>
<td>augmentation</td>
<td>86</td>
</tr>
<tr>
<td>aPC overexpression</td>
<td>streptozotocin-ind. diabetes</td>
<td>attenuation</td>
<td>46</td>
</tr>
<tr>
<td>HGF gene transfer</td>
<td>aristolochic acid</td>
<td>attenuation</td>
<td>87</td>
</tr>
</tbody>
</table>
velop largely independent of inflammatory events. Nevertheless, if the observations with bleomycin injured mice can be transferred to human diseases, this would be very suggestive of a therapeutic efficacy of pro-fibrinolytic or anticoagulatory strategies.

Early clinical trials are either under way or planned to assess the role of pro-fibrinolytic or anticoagulatory strategies in IPF: Systemically applied FXa antagonists, inhalative heparin or warfarin therapies are under investigation. Following the more downstream signaling concept, blockade of PAR-signaling as well as administration of HGF may represent alternative approaches, although some questions will have to be answered before entering clinical trials. For example, it is unclear which type of PAR (PAR-1 or PAR-2 or both) is the most decisive one for development of fibrosis under clinical conditions, and it is also not known if application of u-PA would be more effective as compared to an administration of HGF alone (suggestive of an additional working principle apart from HGF activation).

As for kidney fibrosis, matters are somewhat more complex. The relative role of fibrin deposition per se remains largely unknown, because corresponding studies as performed in the lung (41, 42) have not yet been undertaken. Likewise, the role of procoagulant factors is not clear, as experimental data are scarce. In a study in rabbits with experimental glomerulonephritis (90), anti-coagulation induced by heparin application did not protect from crescent formation. Similar to the lung, PAI-1 has been (almost unequivocally) identified as a central regulator of renal fibrosis, regardless of the model (Tab. 1). In this respect, absence of PAI-1 turned out to be protective in a variety of renal fibrosis models, whereas overexpression resulted in increased fibrosis. Similarly to the lung, PAI-1 has been identified as a therapeutic target for treatment of fibrotic kidney diseases in several studies (37). In view of the relative role of the plasminogen activators and plasminogen, however, there is ongoing discussion and controversial data exist. Pending on the model, t-PA or plasminogen have either been shown to exert beneficial or detrimental effects on renal fibrosis. In view of plasminogen, a direct activation of PAR-1 has recently been suggested to underlay a profibrotic action in the obstructive nephropathy model (89).

Genetic ablation of the u-PA or the uPA-receptor gene seemed not to significantly alter the course of obstructive nephropathy or crescent anti-GBM nephritis (74, 85) with one exception (86). Interestingly, therapeutic elevation of circulating HGF protected from development of renal fibrosis in response to aristolochic acid (87), thus reproducing the beneficial effects as seen in the bleomycin model of lung fibrosis. As suggested in a recent review (37), one reason for the rather confusing results in view of the plasminogen activators, uPA-receptor and PAI-1 may be related to the fact that these serine proteases and serpins are regulated in a cell- and compartment- (glomerular versus tubulo-interstitial) specific fashion and that differences in the readout parameters of renal fibrosis may – to some extent – be caused by this differential distribution and regulation of single factors.

### Conclusion

Alterations of the haemostatic balance are frequently encountered in lung or renal fibrosis. In the lung, all existing data have been obtained in one and the same model and are highly suggestive of a profibrotic role of the procoagulant serine proteases TF/VII, FX and thrombin and an anti-fibrotic role of the u-PA/plasmin system. At least to some extent, PAR-1/2- and HGF-activation, respectively, offer underlying signaling events. However, in how far the data obtained in one single model of lung fibrosis can be transferred to the clinical situation remains open. This may be answered by ongoing or planned clinical trials. In the case of renal fibrosis, only PAI-1 has been identified as important modifier of renal fibrosis regardless of the model used. It may turn out as a target in future clinical trials.

### Acknowledgement

The authors have been supported by the Deutsche Forschungsgemeinschaft (Bonn, Germany) in the Clinical Research Group 118 (Pathomechanism and Therapy of Lung Fibrosis) and the Excellence Cluster „Cardiopulmonary System“.

### References


56. Swaisgood CM, French EL, Noga C et al. The de- velopment of bleomycin-induced pulmonary fi-


Correspondence to:
Prof. Dr. med. Andreas Günther
University of Giessen Lung Center, Klinikum Hohe Straße 36, 35392 Giessen, Germany
E-Mail: andreas.guenther@uglc.de