Potential novel targets: Protease-activated receptors in idiopathic pulmonary fibrosis
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Chapter 1

General introduction and outline of the thesis
General Introduction

Idiopathic pulmonary fibrosis (IPF) is the most frequent diffuse fibrosing lung disease with unknown cause of pulmonary fibrogenesis\textsuperscript{1,2}. It was first described as “chronic pneumonitis” in the late 19\textsuperscript{th} century based on a vague clinical characterization of inflammatory exudation and fibrosis of the lung. Although several similar cases were documented during the following decades, there was no clear and definite classification of this rare pulmonary disease for many years. In 1944, a more detailed description of the clinical and pathological features of a disease referred as “acute diffuse interstitial fibrosis” was provided, which helped greatly on identification of further cases of IPF. Due to the increasing recognition of IPF, the understanding of the pathogenesis of IPF has greatly improved in the past decades. However, it did not remarkably prolong the survival of IPF patients\textsuperscript{3}. Possible targets for novel therapeutic interventions for IPF are thus eagerly awaited for. In this thesis, pharmacological inhibition of blood coagulation factor receptors for the treatment of pulmonary fibrosis is evaluated and based on the obtained results the underlying mechanisms by which the receptors may influence pulmonary fibrosis were studied.

Idiopathic pulmonary fibrosis

The understanding of processes underlying lung repair after injury are important to appreciate the development of pulmonary disease. The architecture and physiology of the lung is complex as evident from the fact that more than forty cell types are involved in the development and repair of the lung\textsuperscript{4}. Of these cell types, lung epithelial cells are key components as they form the structural barrier between the capillaries and the alveoli. Type I alveolar epithelial cells (AECI) are the most abundant pulmonary epithelial cells with attenuated cytoplasm and minimal thickness, facilitating gas exchange such as oxygen and carbon dioxide. Type II AECs (AEC II), which take up around 10\% of the alveolar surface area, are normally responsible for the production and secretion of surfactant but can also act as facultative progenitors as they are capable of replacing themselves and differentiating into AECI after injury\textsuperscript{4}. Under pathologic conditions AEC II cells are activated and promote fibroblast accumulate in injured areas where they differentiate into myofibroblasts that secrete collagen and other extracellular matrix (ECM) proteins.
IPF is a lethal disease, characterized by (myo)fibroblast proliferation and excessive ECM formation leading to destruction of the lung architecture\textsuperscript{2,5}. The lungs of IPF patients become stiff and lose their ability for gas exchange and patients usually suffer from progressively worsening cough and dyspnea caused by decreasing lung compliance\textsuperscript{6}. The prognosis of IPF is devastating with a median survival of 3 years after diagnosis and a mortality rate that exceeds many types of cancer\textsuperscript{7}. IPF occurs in middle-aged and older adults with an age range from 55–75\textsuperscript{2}. The prevalence of IPF rises in the world population and deaths attributed to IPF still continues to increase in the 21st century. There are approximately 14,000 persons diagnosed with IPF each year in the U.S and >5000 new cases to be expected in the U.K\textsuperscript{8,9}. Although the etiology of IPF remains elusive, several genetic and environmental risk factors predispose patients to IPF. For example, mutations in the TERT or TERC genes are causal genetic defects in over 15% of pulmonary fibrosis families\textsuperscript{10}. Other than genetic mutations, smoking, viral or bacterial infections, exposure to therapeutic or environmental toxins are also risk factors for IPF\textsuperscript{11-13}.

Although considerable effort has been made to develop pharmacologic therapies for IPF in order to meet the urgent needs of patients, the progress in patient care is modest. IPF was originally considered a chronic inflammatory response caused by lung injury\textsuperscript{3,14} and IPF patients were consequently treated with anti-inflammatory agents like prednisone or with cytotoxic agents such as azathioprine\textsuperscript{15,16}. Although these agents may help to improve symptoms and give patients pain relief to some extent, they have shown little clinical efficacy in IPF\textsuperscript{15}. The failure of anti-inflammatory therapy in diminishing IPF progression or delaying death boosted research for alternative antifibrotic therapies. Currently, more than 20 agents have been tested in clinical trials for the treatment of IPF only a few have completed phase 3 clinical trials\textsuperscript{17}. Of the four completed phase 3 trials, the ones with Bosentan and Ambrisentan were negative as the primary end-points (delaying worsening of respiratory function and/or death) were not met, whereas the trails analyzing pirfenidone and nintedanib showed effective antifibrotic effects of these drugs in IPF patients. Both of these latter drugs were officially approved in 2014 by the U.S. Food and Drug Administration (FDA) for the treatment of IPF\textsuperscript{18}. Indeed, both pirfenidone and nintedanib treatment slow down the decline of lung function and reduce disease progression in patients with IPF\textsuperscript{19,20}. However, these drugs are associated with side effects and do not
stop nor reverse disease progression. Therefore, lung transplantation is still the most effective treatment for IPF, not only prolonging patient survival but also enhancing the quality of life. Importantly however, not all patients can undergo lung transplantation due to strict requirements for patients’ health condition and limited supply of donor lungs. Novel therapeutic targets and strategies are thus still eagerly awaited for throughout the world and such targets will only become available due to a comprehensive and integrated understanding of the pathogenesis of IPF.

**General mechanisms underlying IPF**

As already touched upon, IPF was originally thought to be the result of chronic inflammation in response to lung injury with unknown cause. However, a growing body of evidence obtained in the past years shows that inflammation is not a vital event in the pathogenesis of IPF. As mentioned above, anti-inflammatory therapies generally present a disappointing outcome in patients with IPF, which is in line with the recent notion that inflammation is hardly observed in histopathological samples of advanced IPF. In addition, clinical analysis of inflammatory markers did not correlate with disease severity in IPF patients. Furthermore, animal studies show that epithelial injury caused by repeated irritations of the lung is sufficient to induce pulmonary fibrosis in the absence of inflammatory responses.

More recent hypotheses suggest that the pathogenesis of IPF is independent of inflammation. Instead, it is proposed that wound repair signaling pathways triggered by repeated alveolar epithelial injury in some way become dysregulated and aberrant, leading to progressive and irreversible fibrosis over time. In the early stages after lung tissue damage, the coagulation cascade is activated thereby triggering platelet activation and the subsequent local secretion of soluble mediators resulting in increased vascular permeability and further damage to the basement membrane. Concurrently, injured epithelial or endothelial cells produce and release excessive inflammatory mediators, inducing the subsequent entry of leucocytes through the disrupted basement membrane. Recruited leucocytes such as macrophages, secrete profibrotic cytokines (e.g. TGF-β, IL-13 and IL-1) and they contribute to a series of events leading to myofibroblast accumulation. Myofibroblasts are highlighted as the main effector cell in fibrosis as they are the major source of ECM components, like collagen and fibronectin.
Profibrotic cytokines together with other growth factors such as platelet-derived growth factor (PDGF) induce resident lung fibroblasts to proliferate, migrate and differentiate into myofibroblasts. Myofibroblasts may also be derived from either fibrocytes from the bone marrow that are attracted by CXCL12 to the injured site\textsuperscript{2,5,29}, or epithelial cells via epithelial–mesenchymal transition (EMT)\textsuperscript{24,30}. Finally, in the tissue remodeling phase, provisional ECM formation promotes wound healing and restores tissue structure. Fibrosis develops when wound repair processes keeps going on because of repeated injury or when any part of the process fails to function normally, changing the provisional ECM formation at the site of tissue injury into a permanent scar. Thus, the initiation and maintenance of fibrosis is a very complex process that involves many effector cells and mediators and identifying the critical contributors may provide new insight into treatment of IPF.
Coagulation and protease-activated receptors (PARs)

The coagulation cascade is responsible for fibrin formation at sites of damaged blood vessels thereby preventing blood loss. Coagulation is initiated instantly upon tissue injury and the coagulation pathway is triggered by the exposure of tissue factor (TF) to plasma Factor VII (FVIIa), facilitating the formation of the TF-FVIIa complex. This complex, either directly or via the intrinsic FIX/FVIII pathway, catalyzes the activation of factor X (FX) to FXa, which together with factor V (FVa) converts pro-thrombin to thrombin. Minute amounts of thrombin subsequently amplify the pathway via activation of FVIII and IX, generating more FXa and thrombin. Finally, thrombin turns soluble fibrinogen into insoluble fibrin strands, leading to a blood clot. Once formed, thrombin also induces a negative feed-back loop to prevent excessive coagulation. After binding to thrombomodulin, thrombin activates protein C (PC), which in turn inactivates factors Va and VIIIa thereby preventing further thrombin formation (Figure 2).
Interestingly, coagulation proteinases also contribute to a striking range of pathophysiological functions independent of fibrin formation. The cellular effects of coagulation factors are mediated by protease-activated receptors (PARs), which are seven-transmembrane G protein-coupled receptors. As opposed to classical GPCRs that are activated by ligand binding, PARs are irreversibly activated by proteolytic cleavage. Protease binding to PARs and subsequent cleavage of the extracellular N-terminus reveals a novel tethered ligand that folds back over the receptor to trigger their transmembrane signaling to intracellular G proteins. To date, there are four known members of the PAR family of which PAR-1 was the first to be discovered in the early 1990s. After intensive studies centered on revealing how thrombin influences cellular responses, PAR-1 was originally identified on human platelets and termed “the cloned thrombin receptor” by Coughlin and co-workers. The second member of PAR family (i.e. PAR-2) was identified in 1994 by Nystedt, who cloned a mouse genomic DNA sequence encoding a protein that shares the identical structure and activation mechanism with PAR-1 but has a different tethered ligand pharmacology from PAR-1. Later on, PAR-3 and PAR-4, the other two thrombin receptors, were also identified by the same group which discovered PAR-1. All four PARs can be activated by individual coagulation factors but they have their distinct N-terminal cleavage sites and tethered ligand sequences which trigger different functional responses. Thrombin is a major activator of PAR-1, PAR-3, and PAR-4, whereas PAR-1 can also be activated by FXa and APC. As for PAR-2, it is the target of both FXa and the TF-FVIIa complex (Figure 2). In addition to direct interactions with receptors, thrombin is also capable of trans-activating PAR-2 by generating the tethered ligand of PAR-1, which in turn binds to adjacent PAR-2. Apart from proteinases, synthetic agonist peptides designed according to the sequence of the cleaved N-terminus can activate PARs in the absence of proteases-induced cleavage (SFLLRN for PAR-1, SLIGKV for PAR-2 and GYPGKF for PAR-4). PAR-3, however, cannot be activated by synthetic peptides and it appears that PAR-3 may act as a cofactor to enhance the affinity of PAR-4 for thrombin-mediated activation or PAR-3 may form heterodimers with PAR-1 to signal together, rather than signaling by itself.

Although blood coagulation factors are the archetypal proteases activating PARs, it is now well established that multiple other proteases can activate individual PARs with different affinity and triggering specific responses via biased agonist signaling. Besides thrombin, PAR-1 can for instance be activated by matrix
metalloproteinases, kallikreins and Granzymes. PAR-2 may also be activated by trypsin, and trypsin-like peptidases such as mast cell tryptase or matriptase.

Coagulation and PARs in the pathogenesis of IPF
Fibrin deposition is a key histological feature of IPF and coagulation activation in response to tissue injury is increasingly recognized to be a critical contributor in the pathogenesis of fibrotic lung disorders. Indeed, patients with IPF are more likely to have a hypercoagulable state and prothrombotic mutations are overrepresented in IPF patients as compared to the general population. In line, increased thrombin activity is considered as one of the characteristic features of pulmonary fibrosis. Gene expression and protein levels of coagulation factors, such as TF, FVII, FXa and thrombin, are increased in patients with progressive IPF and individual coagulation factors exert pro-fibrotic cellular effects through PARs. Thrombin, as the best-known profibrotic coagulation factor, activates PAR-1 leading to myofibroblast accumulation through activating resident lung fibroblasts, promoting epithelial-mesenchymal transition of lung epithelial cells and/or by inducing differentiation of fibrocytes. Thrombin is also a potent inducer of several profibrotic cytokines, including TGF-β, connective tissue growth factor and PDGF. Moreover, FXa induces pro-fibrotic effects of fibroblasts via PAR-2, resulting in the secretion of cytokines like monocyte chemoattractant protein (MCP)-1, interleukin (IL)-6 and TGF-β. Additionally, FXa induces myofibroblast differentiation and TGF-β activation in a PAR-1 dependent manner. More recently, FVIIa was shown to stimulate proliferation and ECM production of human lung fibroblasts via PAR-2 and these proliferative effects of FVIIa were considerably potentiated in the presence of TF, suggesting that the PAR-2/TF/FVIIa axis may contribute to the progression of IPF. Furthermore, FXIIa, a coagulation factor from the intrinsic pathway, strongly stimulates migration of human lung fibroblasts (HLF) derived from IPF patients. More interestingly, HLF from IPF patients exhibit an enhanced FXIIa-binding capacity, suggesting FXIIa contributes to fibrogenesis. The potential importance of coagulation factors in IPF is further underscored by the fact that inhibiting coagulation limits pulmonary fibrosis in preclinical experimental animal models.

In contrast to the pro-coagulant coagulation factors, the endogenous anticoagulant activated protein C (APC) exhibits a protective effect in lung injury. Endogenous APC inhibits infection-induced coagulation activation and APC over-
expression modifies neutrophil recruitment during experimental pneumococcal pneumonia. In addition, APC administration attenuates the expression of inflammatory mediators in acute lung injury induced by hyperoxia and protects mice against sepsis. Interestingly, decreased protein C activation is actually associated with the severity of IPF at diagnosis and APC treatment is proposed to protect mice from bleomycin-induced lung fibrosis.

Anticoagulant treatment in IPF
Due to the fact that the coagulation cascade seems to be instrumental for the etiology in IPF and anticoagulants have proven efficacious in lung fibrosis models, inhibition of the coagulation cascade may be a good strategy for the treatment of IPF. This hypothesis was first addressed in a trial with 56 IPF patients. In this study, patients received prednisolone alone or prednisolone in combination with anticoagulant therapy. The anticoagulants included oral warfarin (an anticoagulant that inhibits the vitamin K–dependent synthesis of thrombin, FVII, FIX, FX and protein C) in an outpatient setting and low-molecular-weight heparin for rehospitalized patients with severely progressive respiratory failure. Anticoagulant therapy showed a beneficial effect on survival in patients with IPF. However, the IPF population in this study was criticized not to be representative, because all patients were nonsmokers while IPF is strongly associated with smoking. In addition, it may contain a misclassification bias, which may have resulted in an overestimated efficacy of the anticoagulant therapy. Later on, heparin inhalation was tested in 20 IPF patients for safety only in an open-label exploratory pilot study. The treatment appeared to be safe and well tolerated in IPF patients, however, whether it will improve any disease related symptoms still needs to be investigated. Recently, a placebo-controlled trial of warfarin was performed including 145 IPF patients. In contrast to the aforementioned study, warfarin did not show a benefit in patients with progressive IPF. Instead, it increased the risk of mortality and hospitalization, which was not caused by major and/or minor bleeding complications. This finding was later supported by a retrospective cohort study, showing that warfarin deteriorated respiratory status and decreased survival of IPF patients. Although coagulation factors seem to play center roles in the pathogenesis of IPF, general inhibition of the coagulation cascade may not be an ideal approach to combat IPF. It may be better to target individual coagulation factors and/or their specific receptors modifying fibrotic disease.
Targeting PARs in fibrotic diseases

PAR-1 and PAR-2 are recently highlighted as potential targets for therapeutic interventions in treating several fibrotic disorders. Activated PARs mediate a number of pathophysiological pathways involved in inflammatory and fibrotic diseases of different organs throughout the body, including brain, lung, heart, liver, kidney and gastrointestinal tract. For example, PAR-1 receptor gene polymorphisms significantly influence the progression of liver fibrosis and the expression of PAR-1 is elevated during acute and chronic human liver injury\textsuperscript{68-69}, whereas PAR-2 activation leads to increased TGF-\(\beta\) production and induces a profibrogenic phenotype in human hepatic stellate cells\textsuperscript{70}. Moreover, PAR-2 regulates PAR-1-driven hyperplasia in response to arterial injury and overexpression of PAR-2 in mice induces cardiac fibrosis, inflammation and heart failure\textsuperscript{71,72}. In the context of brain diseases, PAR-1 mediates neurotoxicity induced by Granzyme B released from T cells during neuro-inflammatory disorders, whereas upregulation of PAR-2 has been observed during neuro-inflammation in the brain tissue from patients with HIV-1-associated dementia\textsuperscript{73,74}. During renal fibrotic disorders, PAR-1 activation by plasmin(ogen) triggers the induction of EMT, while PAR-2 deficiency reduces unilateral ureteral obstruction-induced renal tubular injury and fibrosis\textsuperscript{75,76}. Furthermore, both PAR-1 and PAR-2 modulate radiation-induced intestinal fibrosis\textsuperscript{77,78}. Next to vital organs in the body, PAR-1 and PAR-2 also mediate pro-inflammatory and pro-fibrotic cellular events in important tissues, such as the skin and the joint\textsuperscript{79-80}.

With respect to lung injury and pulmonary fibrosis, accumulating evidence suggests that both PAR-1 and PAR-2 induce pro-inflammatory and pro-fibrotic processes that aggravate disease progression. PAR-1 and PAR-2 are both widely expressed on many different pulmonary cell types like (among others) fibroblasts, macrophages, epithelial and endothelial cells. PAR-1 activation enhances inflammation in the pulmonary epithelium, by mediating macrophage/monocyte recruitment, excessive cytokine release and endothelial barrier disruption\textsuperscript{81,82}. PAR-1 also promotes profibrotic effects by facilitating TGF-\(\beta\) activation, inducing the differentiation of fibroblasts into myofibroblasts and stimulating ECM synthesis\textsuperscript{55,83}. In addition, PAR-1 seems to synergize with PAR-3 to mediate epithelial-mesenchymal transition of alveolar epithelial cells\textsuperscript{84}. The importance of these in vitro findings is emphasized by the fact that genetic ablation of PAR-1 limits bleomycin-induced acute lung inflammation and fibrosis, as evident from
reduced total collagen levels in the lung in combination with decreased levels of proinflammatory and profibrotic mediators, such as TGF-β, IL-6 and MCP-1. More clinically relevant, PAR-1 expression is increased within fibroproliferative and inflammatory foci in IPF patients.85

Similar to PAR-1, PAR-2 also plays a crucial role in promoting pulmonary inflammatory and fibrotic responses. PAR-2 activation leads to endothelial barrier dysfunction and vascular permeability during acute lung injury.86 Activated PAR-2 also enhances inflammatory signaling in airway epithelial cells by increasing cytokine production, such as IL-8, and PAR-2 deficiency reduces allergic lung inflammation in mice.87,88 Moreover, PAR-2 triggers fibroproliferative responses such as proliferation, migration, differentiation into myofibroblasts and release of profibrogenic cytokines (e.g. TGF-β) in human and murine fibroblasts (Figure 3). The absence of PAR-2 affords protection from bleomycin-induced pulmonary fibrosis, as evident from a reduction in the extent and
### Table 1. Application of antagonists of PARs in different fibrotic or inflammatory disorders.

<table>
<thead>
<tr>
<th>PARs</th>
<th>Antagonists</th>
<th>Organs/Tissue</th>
<th>Models</th>
<th>Key results</th>
</tr>
</thead>
<tbody>
<tr>
<td>PAR-1</td>
<td>SCH79797</td>
<td>Heart(^{50})</td>
<td>ischemia-reperfusion injury</td>
<td>Limited left ventricular (LV) dilation and improved LV systolic function of the reperfused myocardium</td>
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<td></td>
<td></td>
<td>Kidney(^{52})</td>
<td>ischemia-reperfusion injury</td>
<td>Significantly attenuated kidney damage by improving serum creatinine</td>
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<td></td>
<td></td>
<td>Intestine(^{94})</td>
<td>ischemia-reperfusion injury</td>
<td>Intestinal myeloperoxidase and adhesion molecule expression were significantly decreased</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Brain(^{95})</td>
<td>Surgically induced brain injury (SBI)</td>
<td>Reduced secondary brain injury by decreasing both brain edema and apoptosis</td>
</tr>
<tr>
<td></td>
<td>SCH602539</td>
<td>Intestine(^{93})</td>
<td>Chronic intestinal radiation fibrosis</td>
<td>Markedly reduced early intestinal radiation injury, but had no effect on the level of delayed intestinal radiation fibrosis</td>
</tr>
<tr>
<td>PAR-2</td>
<td>P1pal-12S</td>
<td>Lung(^{56})</td>
<td>CLP-induced sepsis</td>
<td>Early administration significantly increased survival rate</td>
</tr>
<tr>
<td></td>
<td>RWJ-56110</td>
<td>Liver(^{96})</td>
<td>Bile duct ligation-induced fibrosis</td>
<td>Reduced liver type 1 collagen mRNA and protein expression, as well as hepatic and urinary excretion of hydroxyproline</td>
</tr>
<tr>
<td></td>
<td>NDGA</td>
<td>Skin(^{101})</td>
<td>Oxazolone-induced atopic dermatitis</td>
<td>Rebuilt skin barrier and increased transepidermal water loss recovery</td>
</tr>
<tr>
<td></td>
<td>GB88</td>
<td>Joint(^{97})</td>
<td>Collagen-induced arthritis</td>
<td>Reduced pathological and histopathological changes (i.e. edema, macrophage invasion, mast cell degranulation, etc.)</td>
</tr>
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<td></td>
<td></td>
<td>Colon(^{98})</td>
<td>PAR2 agonist or 2,4,6-trinitrobenzenesulfonic acid-induced Colitis</td>
<td>Reduced mortality and pathology (including colon obstruction, ulceration, wall thickness, and myeloperoxidase release)</td>
</tr>
<tr>
<td></td>
<td>ENMD-1068</td>
<td>Joint(^{99})</td>
<td>Intra-articular carrageenan/kaolin injection induced joint swelling</td>
<td>Dose dependently attenuated joint inflammation</td>
</tr>
<tr>
<td></td>
<td>FSLLRY-amide</td>
<td>Lung(^{102})</td>
<td>Monocrotaline induced pulmonary hypertension (PH)</td>
<td>Reversed established PH in hypoxia–exposed mice</td>
</tr>
<tr>
<td></td>
<td>P2pal-18S</td>
<td>Pancreas(^{100})</td>
<td>Retrograde intraductal bile acid infusion induced biliary pancreatitis</td>
<td>Reduces the severity of biliary pancreatitis</td>
</tr>
</tbody>
</table>
severity of fibrotic lesions and diminished collagen expression. Further, PAR-2 mRNA and protein levels are elevated in lungs of IPF patients as compared to healthy donor lungs and the increased PAR-2 expression is associated with hyperplastic alveolar type II cells and fibroblasts/myofibroblasts. Noteworthy, unlike PAR-1, which expression is already relatively high in normal lung fibroblasts, PAR-2 expression is low in quiescent lung fibroblasts but may considerably increase under inflammatory and fibrotic conditions.

It is thus tempting to speculate that inhibition of PARs may be a promising therapeutic option in the treatment of IPF. In recent years, pharmacological inhibition of PAR-1 or PAR-2 has proven efficacious in different inflammatory and fibrotic preclinical models (Table 1). PAR-1 inhibition with SCH79797 protects mice from ischemia-reperfusion induced heart, kidney and intestine injury, as well as surgically induced brain injury. Blocking PAR-1 with pepducin-based inhibitors at early time points of cecal ligation and puncture (CLP)-induced sepsis significantly prolonged survival. Furthermore, PAR-1 inhibition with RWJ-56110 limits experimental liver fibrosis, as evident from reduced collagen production in the liver. Compared to PAR-1, pharmacological inhibition of PAR-2 is more widely applied in inflammatory models. Targeting PAR-2 ameliorated pathological changes in inflammatory colon and joint diseases. Moreover, PAR-2 inhibition attenuated the severity of experimental biliary pancreatitis and blocked PAR2-mediated inflammatory signaling pathways in atopic dermatitis. Additionally, PAR-2 inhibition by FSLLRY-NH2 reduced the number of fully muscularized vessels and reversed established pulmonary hypertension in a hypoxia mouse model.

Overall, given the critical role of PARs in mediating pro-inflammatory and pro-fibrotic responses on different cell types, pharmacological PAR-1 and PAR-2 inhibition may benefit a broad range of (PAR-dependent) disorders, like among others IPF.
Aim and outline of this thesis

This thesis aims to explore the efficacy of pharmacologically targeting PAR-1 and PAR-2 in experimental lung fibrosis and intents to obtain more insight on the pathogenesis of pulmonary fibrosis. We show that specific pepducin-based inhibitors of PAR-1 (Chapter 2) and PAR-2 (Chapter 3) are able to limit lung fibrosis when administered before and after induction of fibrosis by bleomycin instillation. After showing that both PAR-1 and PAR-2 inhibition limits pulmonary fibrosis, we hypothesized that the simultaneous inhibition of PAR-1 and PAR-2 would be superior to targeting either receptor alone in pulmonary fibrosis. However, the experiments described in Chapter 4 refute this hypothesis and instead show that the pro-fibrotic effects induced by PAR-1 require the presence of PAR-2. Next, we sought to provide mechanistic explanations for observations obtained in chapter 2 and we identified novel mechanisms by which PAR-1 stimulation on different cell types can contribute to pulmonary fibrosis (Chapter 5). Finally, in Chapter 6 we study the role of the endogenous anticoagulant factor APC in bleomycin-induced pulmonary fibrosis. We end the thesis with Chapter 7, a summary and general discussion chapter providing the overall conclusions and profound implications derived from this thesis.
CHAPTER 1

Reference


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CHAPTER 1


