Potential novel targets: Protease-activated receptors in idiopathic pulmonary fibrosis
Lin, Cong

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Chapter 7

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SUMMARY

Despite the high standards of modern medical care and the fast emerging anti-fibrotic agents, mortality rates for idiopathic pulmonary fibrosis (IPF) are still increasing over the years. At a conservative estimate, there are approximate 0.2 million patients suffering from IPF living in the Europe1.

In this thesis, Chapter 1 gives an overview of IPF and introduces coagulation factors and their receptors, PARs, as potential contributors to fibrotic processes. Anticoagulant treatment in IPF is also discussed and to the overall conclusion of chapter 1 is that inhibition of PARs may be specific and effective therapeutic interventions for fibrotic disorders. We therefore set out to address this hypothesis and we show that pharmacological targeting of PARs with pepducin P1pal-12 (PAR-1 antagonist in Chapter 2) or P2pal-18S (PAR-2 antagonist in Chapter 3) effectively blocks PAR-1 or PAR-2 induced pro-fibrotic responses in fibroblasts and limits bleomycin-induced pulmonary fibrosis, even when administered 7 days after the induction of fibrosis. Importantly, in the experimental bleomycin model, pulmonary fibrosis is not completely abolished in mice that harbor deficiencies for either PAR-1 or PAR-2. Therefore, in Chapter 4, we studied the simultaneous inhibition of PAR-1 and PAR-2 in pulmonary fibrosis and we show that treatment of PAR-2 deficient mice with the PAR-1 antagonist P1pal-12 did not further reduce bleomycin-induced lung fibrosis as compared to wild type mice treated with P1pal-12 or PAR-2 deficient mice. Interestingly, PAR-1-induced pro-fibrotic effects in vitro are abolished in the presence of the specific PAR-2 inhibitor. We thus postulate that the pro-fibrotic effects induced by PAR-1 require the presence of PAR-2. Chapter 5 shows that PAR-1 on macrophages potentiate their recruitment towards injured lung epithelial cells. Once recruited, macrophages secrete a PAR-1 agonist, most likely FXa that acts upon fibroblasts leading to the production and activation of latent TGF-β which subsequently drives fibroblast migration, differentiation into myofibroblasts and ECM deposition. Finally, in Chapter 6 we addressed the importance of the endogenous anticoagulant system, especially APC, in the development of pulmonary fibrosis. We show that high endogenous APC levels reduce bleomycin-induced pulmonary fibrosis and this reduction in pulmonary fibrosis may be explained by the inhibitory effect of endogenous APC on macrophage recruitment during the fibrotic phase.
GENERAL DISCUSSION

With the research performed in this thesis, we sought to assess the potential clinical relevance of targeting PARs in pulmonary fibrosis and to identify potential effectors and mechanisms which contribute to the pathogenesis of IPF.

Most importantly, we show that pharmacological inhibition of PARs, even starting after the onset of fibrosis, affords protection against bleomycin-induced pulmonary fibrosis. As current treatment options for IPF are limited\(^2\), these findings may be particularly interesting for future medical interventions in IPF patients. Importantly, PAR-1 inhibition may be feasible on a short notice, as the PAR-1 inhibitor Zontivity just obtained FDA approval for antiplatelet therapy of patients with a previous myocardial infarction or leg ischemia\(^3\). Its application along with standard therapy effectively limits thrombotic cardiovascular events, yet Zontivity increases the rate of moderate to severe bleeding in a selection of patients\(^4\). Although PAR-1 inhibition thus seems an attractive strategy to pursue in IPF, potential bleeding complications should be taken into consideration and patients should be properly monitored.

Based on the notion that PAR-1 seems to drive pulmonary fibrosis in a PAR-2 dependent manner, it is tempting to speculate that blocking PAR-2 may be a better treatment strategy for IPF than using Zontivity. In addition, PAR-2 inhibition does not interfere with platelet activation as does PAR-1 inhibition. Therefore, targeting PAR-2 will not cause bleeding complications and may be the preferred treatment option. Unfortunately, there are no PAR-2 inhibitors currently available for clinical intervention.

PAR-1 is activated by coagulation factor Xa and thrombin, whereas PAR-2 is activated by the tissue factor-FVIIa complex and by factor Xa\(^5\). Consequently, anticoagulant treatment would presumably be effective in IPF as it blocks PAR-1 and PAR-2 simultaneously. Importantly however, anticoagulant therapy in the setting of IPF remains a matter of debate mainly due to the lack of effect of warfarin in recent clinical trials\(^6-7\). Compared to anticoagulant therapy, targeting PARs may be a more efficient (and safe) approach for limiting fibrosis, as PARs are not only activated by coagulation factors. Activation of PARs by several other proteases can also induce profibrotic effects, at least in vitro. For example, Granzyme K is found to induce pro-inflammatory cytokine secretion and lung
fibroblast proliferation through PAR-1 whereas trypsin is well known to trigger fibroproliferative effects via PAR-2.

Our data that PAR inhibition limits pulmonary fibrosis may not only benefit IPF patients but may also have clinical impact in other disorders which involve PARs signaling. Indeed, PARs are suggested to contribute to (among others) heart failure, renal, liver and skin fibrosis. Whether the application of PAR inhibitors will benefit patients suffering from these PAR-related human disorders needs however to be further studied.

The functional relevance of coagulation activation in the pathogenesis of IPF has received much attention, both in preclinical as well as in clinical studies. Surprisingly however, the endogenous anticoagulant pathways, such as APC pathway, received relatively little attention in IPF. In the current thesis, we highlight the potential importance of the endogenous anticoagulant protein C pathway by showing that high APC levels inhibit the progression of bleomycin-induced pulmonary fibrosis. In line, recombinant APC administration also limits bleomycin-induced pulmonary fibrosis, and PC activation is suppressed in IPF patients. Moreover, infusion of APC was recently shown to reduce pulmonary coagulopathy and to decrease lung injury caused by acute respiratory distress syndrome, suggesting APC may also attenuate lung injury in patients with pulmonary fibrosis. Increasing APC levels, either by the administration of APC or zymogen PC, may thus be an alternative strategy for the treatment of IPF. Importantly, recombinant human APC (Xigris) is well known for the treatment of sepsis and recombinant APC would thus be easily available for a clinical trial in IPF. However, the benefit-to-risk ratio of Xigris in patients with sepsis remained controversial for many years. Indeed, the most recent clinical trial on Xigris showed no evidence of benefit for treating patients with severe sepsis or septic shock. Consequently, Xigris was withdrawn from the worldwide market and is not available anymore.

In addition to pinpointing the APC pathway as a potential therapeutic strategy in IPF, our data showing that endogenous APC limits pulmonary fibrosis may also explain (at least in part) the lack of efficacy of warfarin in improving IPF related symptoms. Indeed, warfarin inhibits the activity of all vitamin K dependent coagulation factors including APC. Warfarin would thus limit fibrosis by targeting the pro-coagulant factors but could potentiate fibrosis by inhibiting APC. Therefore,
alternative anticoagulants targeting the pro-coagulant factors without affecting endogenous anticoagulant pathways may have clinical potential.

Overall, we provided several lines of evidence to support the importance of PARs in the pathogenesis of IPF and suggest that targeting PARs, its endogenous agonists or the APC pathway may be novel therapeutic approaches for treating this disease.

REFERENCES

1. http://www.pulmonary-fibrosis.net/


NEDERLANDSE SAMENVATTING

Ondanks de hoge standaard van de moderne medische zorg en de snelle opkomst van anti-fibrotische medicijnen, neemt het sterftecijfer voor mensen met idiopathische longfibrose (IPF) nog steeds toe. Met een algemene schatting zijn er ongeveer 0,2 miljoen patiënten in Europa die lijden aan IPF. In dit proefschrift, geeft Hoofdstuk 1 een overzicht over IPF en introduceert het stollingsfactoren en hun receptoren, protease geactiveerde receptoren (PAR’s), als potentiële mediatoren van fibrotische processen. Antistollingsbehandeling in IPF wordt ook besproken en de algemene conclusie van hoofdstuk 1 is dat de remming van de PAR’s een specifieke en effectieve therapeutische interventie zou kunnen zijn voor fibrotische aandoeningen. Wij hebben er in dit proefschrift dan ook voor gekozen om ons te richten op deze hypothese en we laten zien dat de farmacologische remming van PARs met P1pal-12 (PAR-1 antagonist in hoofdstuk 2) of P2pal-18S (PAR-2 antagonist in hoofdstuk 3) effectief PAR-1 of PAR-2 geïnduceerde pro-fibrotische reacties blokkeert in fibroblasten en verder bleomycine geïnduceerde longfibrose beperkt. Dit laatste is zelfs het geval indien de PAR remmers worden toegediend 7 dagen na de inductie van fibrose. Belangrijk echter, in het experimentele bleomycine model wordt longfibrose niet volledig voorkomen in muizen die geen PAR-1 of PAR-2 tot expressie brengen. Daarom hebben we in hoofdstuk 4 de gelijktijdige remming van PAR-1 en PAR-2 in longfibrose bestudeerd en laten we zien dat de behandeling van de PAR-2 deficiënte muizen met de PAR-1 antagonist P1pal-12 de bleomycine geïnduceerde long fibrose niet verder verminderd in vergelijking met wild type muizen die zijn behandeld met P1pal-12 of PAR-2 deficiënte muizen. Interessant genoeg worden de PAR-1 geïnduceerde pro-fibrotische effecten in vitro geremd door de specifieke PAR-2 inhibitor P2pal-18. We concluderen in hoofdstuk 4 daarom dat de pro-fibrotische effecten geïnduceerd door PAR-1 de aanwezigheid van PAR-2 vereisen. Hoofdstuk 5 laat zien dat PAR-1 op macrofagen hun migratie richting gewonde longepitheelcellen verstert. Eenmaal in de long aangekomen, scheiden de macrofagen een PAR-1 agonist, waarschijnlijk factor Xa, uit die fibroblasten activeert wat leidt tot de productie en activering van latent TGF-β, wat vervolgens fibroblasten aanzet tot tot differentiatie in myofibroblasten en tot de afzetting van extracellulaire matrix productie. Tenslotte wordt er in hoofdstuk 6 ingegaan op de betekenis van het endogene anticoagulante systeem, voornamelijk geactiveerd proteine C (APC), in de ontwikkeling van longfibrose. We laten zien dat hoge endogene APC niveaus bleomycine geïnduceerde longfibrose verlagen en deze vermindering in longfibrose kan worden verklaard door het remmende effect van endogene APC op macrofaag migratietijdens de fibrotische fase.
Acknowledgements

At the very end of this adventurous journey, I would like to thank all the people who contributed to this achievement and accompanied me throughout this experience.

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the future holds greater things for you no matter which path you choose to pave. Dita: I doubted whether I should pick you as my paranimfen, since I was afraid that I will be overwhelmed with your passion and enthusiasm. It turns out that these are the exact things that I want and need. I appreciate you as a good friend because you always show your kindness and caring towards me. I also thank you for putting in effort and time in helping me to prepare all the stuff. As a noodle buddy, thank you for always backing me up with your healthy vegetarian noodle with extra spicy flavor. :D Finally, for all the teletubbies “You have to learn the rules of the game. And then you have to play better than anyone else.” (Albert Einstein) PTG men, PTG… 😊

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# PHD portfolio

Name PhD student: Cong Lin  
PhD period: September 2010 - September 2014  
Name PhD supervisor: Prof.dr.T.van der Poll

## 1. PhD training

### General courses

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<td>Targeting PAR-1 with P1pal-12 pepducin limits pulmonary fibrosis in the murine bleomycin model (oral). European Respiratory Society (ERS). Vienna, Austria</td>
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(Inter)national conferences

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<td>- 9th Lung Science Conference (LSC). Estoril Portugal</td>
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<td>- European Respiratory Society (ERS). Vienna, Austria</td>
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<td>- Congress of the international society on thrombosis and haemostasis (ISTH). Amsterdam, Netherlands.</td>
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2. Teaching

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3. Parameters of Esteem

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List of publications


