Targeting TGFβ signaling pathway in fibrosis and cancer

Karkampouna, S.

Appendix II

Summary

Samenvatting

List of abbreviations

Curriculum Vitae

List of publications

Acknowledgements

Summary

Cancer and chronic fibrosis are devastating diseases of high mortality rate with limited curative therapies available. Both of these diseases may influence the function of one or multiple organs while the extracellular microenvironment is a contributing factor in the pathogenesis of both disorders. Considering the increasing number of cases per year, a better understanding of the biological drivers of these diseases is fundamental in order to develop effective therapeutics. At the molecular level, signaling pathways control cell growth, differentiation or apoptosis during development and adult life of the organism ensuring homeostasis. In a paradox, the same signals are often implicated or even drive disease progression. Environmental or genetic factors have impact on gene functions by direct gene alterations or indirect epigenetic factors that change the function of signaling members. One of the signaling pathways with key regulatory functions in homeostasis, tissue fibrosis and cancer in many organs is the TGFβ/ BMP pathway.

In this thesis we have addressed the role and therapeutic potential of TGFβ/ BMP pathway inhibition using different drug compounds that are currently towards the clinic or being tested in clinical trials. Three distinct types of inhibitors were used; small molecule inhibitors of the ALK4, 5 and 7 TGFβ receptor kinases, an antisense oligonucleotide interfering with ALK5 mRNA splicing and an ALK1 ligand trap; a peptide that contains the extracellular domain of ALK1 fused to Fc and sequesters BMP9 and BMP10. These inhibitors were used in an *ex vivo* human fibrosis model or *in vivo* mouse models of various human diseases (acute liver failure/ liver regeneration, Dupuytren's fibrosis) and cancer (prostate, liver).

Chapter 1 introduces the basic aspects of the TGFβ/ SMAD signaling pathway, including its regulation, crosstalk with other pathways and the role during homeostasis of liver, prostate and connective tissue. Moreover, the impact of TGFβ and BMP deregulation is introduced in relation to common abnormalities; liver wound healing, Dupuytren's fibrosis and prostate tissue malignancies. Advances in drug development have provided researchers with therapeutic strategies with potential clinical application. In this Chapter we address the properties of individual classes of drugs targeting (mainly inhibiting) the TGFβ pathway and their status on ongoing or completed clinical trials.

In Chapter 2 the current knowledge on TGFβ signaling in relation to its contribution in liver regeneration is reviewed. Different aspects are addressed such as embryonic development, the molecular mechanisms regulating hepatocyte and oval cell regeneration, plasticity of hepatic lineages, and induced reprogramming of hepatocytes as cell based therapies for human liver diseases. Repeated tissue injury leads to unrepairable cell regeneration and a shift towards scar tissue formation (fibrosis). The end stage of excess fibrosis in the liver (cirrhosis) is a risk factor for hepatocellular carcinoma development. In order to understand the progression of liver disease further investigation of the mechanisms that balance cell replenishment and fibrosis is needed. Excess activation of TGFβ leads to epithelial cell apoptosis (acute liver failure), epithelial-to-mesenchymal transition, liver cirrhosis and eventually cancer. Thus, *in vivo* inhibition of TGFβ may prove a beneficial therapeutic strategy in liver diseases.

In Chapter 3 we investigated the *in vivo* applicability of a drug compound, the small molecule inhibitor (LY364947) targeting the kinase activity of TGFβ type I receptor (ALK5), in a mouse model of acute injury-induced liver regeneration. Administration of LY364947 in mice that received hepatotoxin carbon tetrachloride (CCl_4) enhanced the proliferation of hepatocytes and recovery of the liver enzyme expression pattern, showing overall improved wound healing compared to mice that did not receive the ALK5 inhibitor. The possible mechanism of this effect is inhibition of the cytostatic effect of TGFβ by downregulation of p21, and/or upregulation of proliferating nuclear antigen and phosphorylation of histone 3.

In Chapter 4 and 5 we addressed the limitations to study the effects of TGFβ inhibition using the current methods available in the field of fibrosis (primary fibroblast cultures). For this we developed novel *ex vivo* culture methodology that allows us to maintain human fibrotic connective tissue from palmar fascia (Dupuytren's fibrosis). The proof-of-principle for this *ex vivo* method is introduced in detail in Chapter 4 along with further applications, such as biochemical and imaging techniques to be used for studying patient-specific pathogenesis and responses to candidate antifibrotic drugs. In Chapter 5, we have used the *ex vivo* culture system to assess the anti-fibrotic potential of TGFβ inhibitors. Antisense oligonucleotide and small molecule kinase inhibitor-mediated ALK5 inhibition diminished the number of collagen-producing myofibroblasts and decreased the expression of extracellular matrix proteins indicating an amelioration of fibrosis *ex vivo*. Such preclinical studies highlight the importance of TGFβ inhibition as monotherapy or in combination with other substances, such as anti-inflammatory agents, for the treatment of fibrosis.

In Chapter 6 we explored the effect of the anti-angiogenesis compound ALK1Fc (ACE-041, Acceleron), which acts on different fronts; tumor microenvironment (matrix and vasculature) and tumor cells. Administration of ALK1Fc exhibited *in vivo* anti-angiogenic and tumor suppressive effects in a primary prostate cancer mouse model established by orthotopic transplantation of human prostate cancer cells. ALK1Fc ligand trap is currently being tested in clinical trials for solid tumor treatment as tumor angiogenesis inhibitor. ALK1Fc reduced the tumor volume of primary prostate cancer (formed by aggressive prostate cancer cell line PC3-M-Pro4) possibly due to reduced tumor vasculature. *In vivo* administration of ALK1Fc acted as anti-angiogenic factor by inhibiting BMP9 binding to its receptor ALK1 on endothelial cells. However, ALK1Fc also indicated a direct effect of BMP9 on tumor cells; BMP9 was found to mediate upregulation of the NOTCH ligand JAGGED, the cancer stem cell marker Aldehyde dehydrogenase 1 (ALDH1A1) and to enhance the proliferation of prostate cancer cells.

In Chapter 7 the findings of this thesis presented in the different chapters are discussed under a common denominator; the inhibition of TGFβ/ BMP signaling in human diseases. Furthermore, the implications of TGFβ inhibitory strategies in basic and clinical research along with future perspectives are discussed. Molecular signatures characteristic of tissue injury often reminisce the expression status of human malignancies. Preliminary data indicate a role for the TGFβ type III receptor CRIPTO in liver regeneration and human hepatocellular carcinoma (Appendix I). Normally, CRIPTO is expressed only during embryonic development. We show that reactivation of CRIPTO takes place in the mouse liver after toxin-induced acute injury. CRIPTO has the potential to reduce SMAD2/3 signaling by directly binding to TGFβ and has been suggested as a mediator of the TGFβ- tumor promoting effect by deactivating the

cytostatic role of TGFβ and activating other cell survival signals. Moreover, elevated protein levels of CRIPTO in liver tissue specimens of human hepatocellular carcinoma suggest a tumor-promoting role of CRIPTO in the liver, which may be of potential diagnostic value as biomarker for human hepatocellular carcinoma.

Collectively, after the discovery of the TGFβ numerous studies have contributed to a holistic and comprehensive view of the molecular properties of all the proteins assembling the pathway and the biological processes that TGFβ is involved in. Given the disease-promoting role of deregulated TGFβ/ BMP signaling the key aspect of current research is therapeutic intervention by targeting pathway activation in a clinical relevant way. Based on our observations, inhibition of TGFβ is promising in many diseases and has additive beneficial effects in combination with other drug compounds, for instance chemotherapeutic, anti-inflammatory, immune modulatory agents depending on the disease setting.
However, TGFβ inhibition is not panacea mainly because of the TGFβ homeostatic role in many organs. Successful modulation of TGFβ requires careful patient stratification and disease staging by using prognostic tools such as biomarkers and patient-specific genetic variation. Current and future development in drug delivery will hopefully enhance the tissue-specific or cell type-specific targeting of drugs against TGFβ pathway members along with precise dosing and timing in a personalized scheme of treatment.