Targeting TGFβ signaling pathway in fibrosis and cancer

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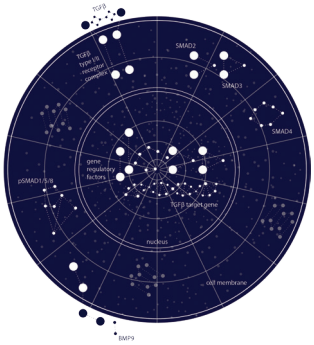

Chapter 7

General Discusion & Future Perspectives

Discussion and Future Directions

TGFβ- the "Many-Faced God"

Cell signaling from members of the TGFβ pathway regulate homeostasis of many organ systems during embryonic development and adult life. TGFβ suppresses the proliferation of epithelial, endothelial and immune cells (tumor suppressor activity) and its action is under constant regulation. When the levels of TGFβ ligands or receptors are abnormally increased or decreased, due to occurrence of genetic mutations that affect gene expression, the cell homeostasis is disturbed; increased apoptosis/ proliferation, immune suppression/ hyperactivation, insufficient wound healing upon injury or excess ECM remodeling. In fact, in human pathological conditions, such as organ fibrosis and cancer, the circulating levels of TGFβ and BMP ligands are often found increased[1]. In particular, in many types of human cancer, TGFβ plays multiple roles[2]; at the initial stages of cancer formation it has tumor suppressing role. Upon establishment of a carcinoma TGFβ is considered to be a tumor promoter factor (mediated via linker phosphorylation of SMAD2/3[3] or non-SMAD pathways); tumor cells deactivate the cytostatic branch of TGFβ and maintain responsiveness to TGFβ-mediated EMT transition, cell motility, migration, differentiation to MFB phenotype and ECM remodeling[2,4,5]. These processes facilitate the spread of tumor cells and metastasis to other organs. Given the increased lethality of cancer patients associated with occurrence of metastasis and the lack of therapeutic treatments for organ fibrosis, several drugs inhibiting the function of TGFβ pathway members have been developed as promising cancer and fibrosis targeted therapies[6].

Summary of Findings

Our studies focused on modulation of TGFβ pathway in homeostasis, human fibrosis and cancer by *in vivo* and *ex vivo* approaches in different organs (liver, prostate, connective tissue). Inhibition of TGFβ/ BMP pathway was performed using an antisense oligonucleotide, a ligand trap and a small molecule kinase inhibitor. Main goal of these different lines of research was to investigate the basic regulatory mechanisms during tissue regeneration (liver), fibrosis (Dupuytren's disease), tumor angiogenesis and cancer (prostate) and potential therapeutic benefits of TGFβ/ BMP inhibition.

The main findings of this thesis are summarized in **Fig.1**.

1. Dynamics in TGFβ signaling activation regulates liver regeneration. Downstream effector SMAD2 mediates cell cycle arrest in hepatocytes and pro-fibrotic effects in HSCs (liver myofibroblasts). Systemic administration of small molecule TGFβ type I receptor kinase inhibitor LY-364947, selective for ALK5 inhibition, reduces basal SMAD2 signaling in hepatocytes, which leads to escape from cell cycle arrest (low p21, high PCNA and phosphorylated histone 3 levels). Hepatocyte proliferation due to ALK5 inhibition leads to improved cell replenishment during acute liver injury in mice and enhanced liver function (Chapter 3).

2. Systemic administration of TGFβ type I receptor kinase inhibitor, LY-364947, is not sufficient to abrogate the fibrogenic response of HSCs in acute, reversible liver injury model (Chapter 3).

3. Human connective tissue from Dupuytren's fibrosis is maintained in *ex vivo* culture for seven-day time period allowing real time study of fibrotic mechanisms by second harmonic generation imaging and three-dimensional reconstruction (Chapter 4).

4. *Ex vivo* delivery of ALK5 inhibitors SB-431542 and ALK5-AON have anti-fibrotic effects in human Dupuytren's fibrosis (Chapter 5).

5. Inhibition of BMP9 by ALK1Fc ligand trap has potential tumor anti-angiogenic effects on human prostate cancer in mouse xenograft model (Chapter 6).

6. ALK1Fc diminishes primary prostate tumor size by inhibiting BMP9 pro-proliferative role directly on tumor cells. BMP9 mediates tumor growth via binding to ALK2 and inducing NOTCH signaling (Chapter 6).

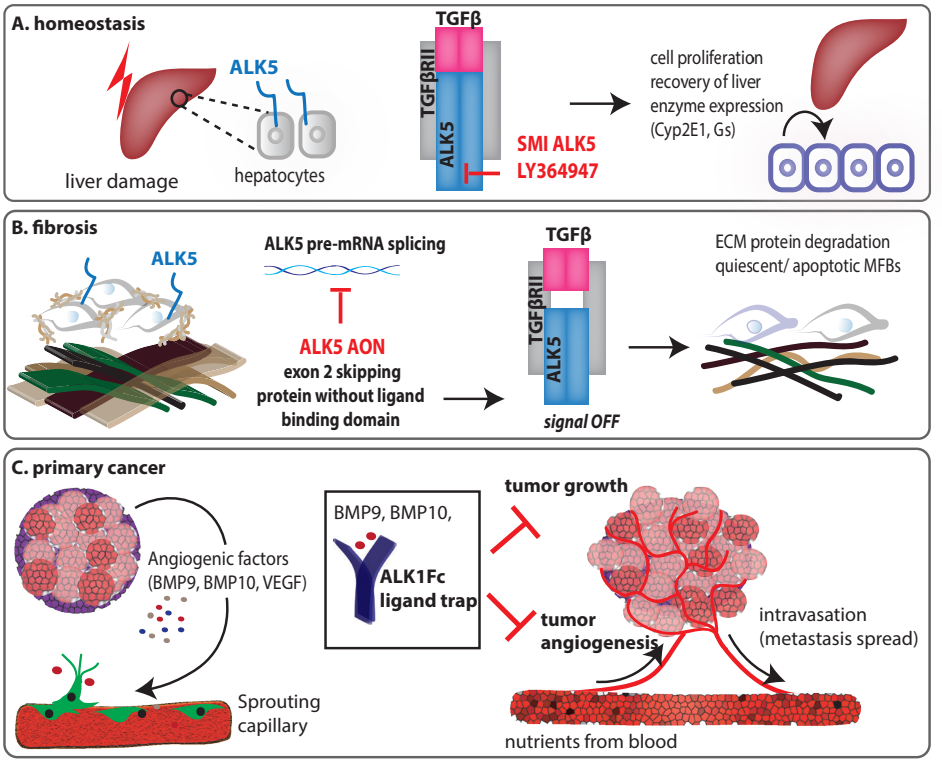

Fig.1. Summary of key findings of this thesis
(A). Liver homeostasis and the effects of *in vivo* administration of small molecule kinase inhibitor LY364947 to inhibit ALK5 signaling (Chapter 3). (B). Connective tissue fibrosis (Dupuytren's disease) and *ex vivo* tissue culture of human specimens (Chapter 4). *Ex vivo* inhibition of ALK5 using antisense oligonucleotide-mediated exon skipping showed anti-fibrotic effects on extracellular matrix protein deposition (Chapter 5). (C). *In vivo* inhibition of tumor angiogenesis and primary prostate tumor growth using the ALK1Fc ligand trap of BMP9/BMP10 in order to interfere with ALK1 and ALK2 downstream signaling.

Modulation of TGFβ- Insight from *In Vivo* Liver Regeneration

Several studies have thoroughly investigated the contribution of hepatic progenitor cells on liver regeneration (reviewed in Chapter 2). These normally quiescent cells (oval cells), reside in the bile duct area and upon chronic injury become activated (ductular reaction) and differentiate to replenish hepatocytes and biliary epithelial cells (cholangiocytes). Various cytokines and cell types (resident and recruited immune or bone marrow cells) play a dynamic role during liver regeneration. Recent studies have increased our understanding of how regeneration is regulated, for instance by transcription factor gene expression alterations[7], contribution of hepatic stellate cells as either source of oval cells[8] or collagen producing MFBs[9], and bile duct (ductular) reaction and its the role was studied by *in vivo* lineage tracing [10]. Preliminary data from this thesis (Appendix I) suggest a potential role for TGFβ type III auxiliary receptor CRIPTO in ductular reaction of oval cells during liver regeneration. However, the origin of these cells requires further characterization, considering that there are various other sources for MFBs[11] during liver injury such as recruited bone marrow derived cells[12], pericytes and endothelial cells[13]. Furthermore, CRIPTO-expressing cells are found in the microenvironment of hematopoietic stem cells[14,15] while CRIPTO can function not only as membranous protein but also in a paracrine or systemic way as a soluble factor[16]. Thus, the origin and identity of CRIPTO-positive cells and their circulating or local role in the liver remain to be elucidated.

Tissue injury causes changes in tissue architecture, different mechanical tension between the connective tissue, pH, altered levels of oxygen and nutrients delivered to cells[17]; all these factors trigger release of latent TGFβ from the matrix proteins and upon activation TGFβ dimer is then able to bind to its receptors on the cell surface and activate the pathway[18]. In Chapter 3, we studied different aspects liver regeneration after experimental injury *in vivo*; response of epithelial cells (hepatocytes), hepatic stellate cells (HSCs, MFBs) and liver functionality. We show that the levels and duration of TGFβ pathway activation are determinants of the regenerative response and that TGFβ has pleiotropic effects in different cell types. *In vivo* systemic administration of small molecule kinase inhibitor (SMI) LY-364947 indicated that decrease of TGFβ/ALK5 pathway in the liver is applicable and has cell-type specific effects. Delivery of SMI and pathway inhibition is more efficient in the hepatocytes and less potent in targeting activated MFBs. It is known that TGFβ has a cytostatic role on hepatocytes (SMAD2-mediated) and a profibrotic and mitogenic role on the MFBs (SMAD3)[19]. In our study, inhibition of ALK5 activity induced proliferation of hepatocytes consistent with the TGFβ-mediated cytostasis. Regarding previous studies showing that soluble TGFβRII delivered *in vivo* reduces fibrosis[20] we expected similar effects with the SMI LY-364947. Our data indicated that deposition of fibrous proteins by MFBs is not inhibited by SMI targeting ALK5. A variety of reasons may account for this response; (1) Kinetics and cell type specific delivery of the SMI[21]. (2) The acute liver injury model used is suitable for the study of regeneration and scar tissue formation (fibrogenesis) but not of pathological fibrosis[22]. Thus, to delineate the antifibrotic effects of LY-364947 different experimental models should be used such as chronic CCl_4 fibrosis model. (3) Alternative non SMAD signaling mechanisms may become activated to compensate for ALK5/SMAD2/3 inhibition[23]. Imatinib, a SMI of c-Abelson (c-ABL) kinase, is an FDA approved drug for chronic myeloid leukemia and also decreases lung fibrosis by inhibiting PDGF and TGFβ-non-SMAD mediated activation of c-ABL[24].

Recent studies using similar SMI for ALK5, such as EW-7197, show reduction of TGFβ-induced reactive oxygen species production and promising anti-fibrotic outcome in liver fibrosis both *in vivo* and *in vitro* [25]. Another inhibitor of ALK5, LY-2157299, has shown safety and efficacy in advanced human cancer such as glioma[26] and in *ex vivo* studies of hepatocellular carcinoma[27,28].

Clinically approved kinase inhibitors are Imatinib (Gleevec), Efitinib (Iressa) and Erlotinib (Tarceva) for cancer treatment have paved the "translational" way of SMIs[29]. The main advantages of SMIs are the high cell permeability due to small molecular weight and decreased toxicity due to rapid metabolism and removal by the renal system. However, *in vivo* evaluation of SMIs requires frequent or periodic administration, since dosage-related and cell type-specific effects are difficult to monitor[30] because the majority of these inhibitors bind to the ATP site of kinases. Cross-inhibition of multiple kinases is a consideration when using ATP inhibitors; however, experimental advances, such as high-throughput screening, have improved the specificity properties for a particular kinase[30-33]. Novel methodologies, in particular in Proteomics and Nanotechnology are needed to further improve the substrate specificity and delivery of SMIs to a particular tissue or cell type that is most likely to benefit from the drug and circumventing toxic effects to other tissues.

Insight on TGFβ-mediated Fibrotic Mechanisms from an *Ex Vivo* Human Model

Fibrosis is a pathological state characterized by the excessive deposition of connective tissue commonly occurring during wound healing and tissue regeneration[34]. It can affect most organ systems and lead to a variety of diseases including liver cirrhosis, pulmonary hypertension, systemic sclerosis and congestive heart failure, representing one of the major medical challenges of our time. To treat fibrotic disease, it is necessary to control the improper wound mechanism process and redirect it towards a pure regenerative/repair process[35]. Design of novel anti-fibrotic therapies can only be achieved after thorough characterization of the disease profile. Our aim was to improve the current methodology available for DD fibrosis in order to better study the molecular pathogenesis. In Chapter 4, we present the development of an *ex vivo* culture method for the maintenance of human fibrotic tissue from Dupuytren's connective tissue disease. This model combines the near-*in vivo*, physiological microenvironment as equivalent to the *in vitro* method where culture conditions and gene expression can be modulated. Given the high rate of recurrence and surgical resection of fibrotic nodules being the main treatment to restore hand functionality, our model provides a patient-specific, preclinical platform for screening of anti-fibrotic compounds using surgical waste material. Fibroblast cell derivation remains the main method to study fibrosis; however it can only provide one-sided information excluding the influence of the microenvironment on cell behavior[36,37]. The advantage of this system is that we circumvent the need for fibroblast cell derivation and instead we study the three-dimensional context of cells and surrounding extracellular matrix[35,38].

Novel *Ex Vivo* Culture Method of Dupuytren's Fibrosis: Preclinical Translation for Human Anti-Fibrotic Drug Screening

DD tissue composition consists mainly of fibroblasts, MFBs, with fewer subset of endothelial, smooth muscle cells and immune cells[39] (**Fig.2**). ALK5 is expressed mainly in MFBs in DD, and not in endothelial cells, which express ALK1[40]. In Chapter 5, using the *ex vivo* model of human fibrotic tissue we demonstrated how interference with the mRNA processing of ALK5 by AON exhibits altered gene expression in favor of fibrosis reversal. We have showed that AONs with two different chemical modifications, Vivo morpholinos and 2'-O-methyl (2'-O-Me), are effectively delivered in tissue and do not cause cell death or toxicity. In our system, the fibroproliferative properties of the disease have been hampered by ALK5 AON treatment, suggesting that partial reduction of the TGFβ signaling is sufficient to "decrease" the fibrotic content of the disease. One possible explanation of the strong anti-fibrotic effect of ALK5 AON observed in DD tissue may be attributed to the occurrence of "flywheel effect" upon downstream proteins. Although, the initial stimulus is removed (ALK5 exon skipping) there seems to be a continuous oscillation of downstream effects, such as protein expression of target genes (collagens, αSMA). Another consideration is that full skipping is not the goal for the treatment of DD especially since partial skipping already has beneficial effects of collagen deposition. Complete abrogation of ALK5 receptor activity might elicit compensatory mechanisms by other signaling pathways with no therapeutic benefit. Therefore, a more subtle and titrable regulation is required, which is one of the major advantages of the AON approach.

In addition, ALK5 kinase activity was effectively inhibited *ex vivo* by kinase inhibitor SMI SB-431542, which is an ATP antagonist and blocks ALK4, -5, -7 kinase activity. In both strategies (SB-431542 and ALK5 AON) expression of fibrous proteins collagens type I and type III was decreased and the number of α-smooth muscle actin (αSMA)-positive MFBs was reduced. Since there was no increase in cell death events in our model, perhaps the existing MFBs were modulated in order to secrete fewer amounts of fibrous proteins. Moreover, degradation of collagen is induced by specialized enzymes MMP1, MMP8, MMP13 and MMP14. MMP13 and MMP14 may indirectly also activate TGFβ (via MMP2 and MMP9) exacerbating fibrosis[41]. MFBs produce high levels of tissue inhibitor of matrix metalloproteases (TIMPs) to promote ECM deposition instead of degradation. Fibrosis resolution is associated with disappearance of MFBs and decrease in TIMP expression[42]. Reduction of fibrotic burden and MFBs in our model may be induced from the inhibition of TGFβ-mediated EMT process[13]. Quiescent fibroblasts are distinguished from activated MFBs by αSMA expression, thus, reduction of αSMA observed in real time due to TGFβ inhibition *ex vivo* (Chapter 5) may indicate reversal of MFBs to a quiescent state.

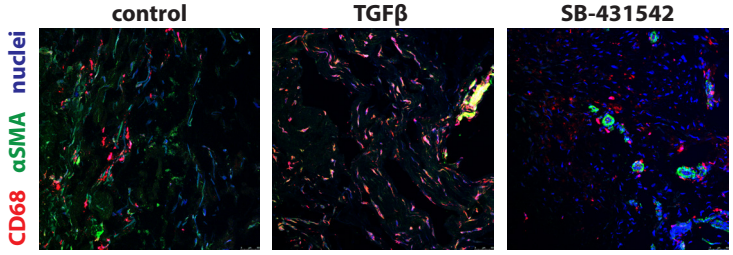

Fig.2. Myofibroblast (αSMA) and macrophage (CD68) expression in the DD tissue
Ex vivo human DD cultures were treated with TGFβ3 (5ng/ml) or SB-431542 (10uM) for 72 hours. Scale bars 50um.

The current opinion is that MFBs once activated and their function is no longer required, they undergo senescence[43] or apoptosis[44]. Another hypothesis is that MFBs may reverse back to a quiescent fibroblast phenotype, which requires further experimental evidence[11,45]. Lineage tracing experiments are required to address whether this hypothesis is valid for DD, with genetic tools applied for tracing MFBs in liver and lung fibrosis *in vivo* (glial fibrillary acidic protein- Cre recombinase (GFAP-Cre)[8], collagen 1 type I (Col-GFP)[11]. and PDGF receptor β (PDGFR β- Cre)[46]. MFBs in several organs are marked by PDGFRβ expression and deletion of αvβ6 integrin in those cells caused a reduction of TGFβ activation and subsequently of fibrosis[46]. Similar effects were observed by small molecule inhibitor targeting αvβ6 integrin[46]. Given the recurrent properties of DD fibrosis the role of stem/ progenitor cells (for instance mesenchymal stem cells) that constantly differentiate towards MFBs might be a valid hypothesis for the origin of fibrosis. Thus, we need to identify the key regulatory proteins that trigger the start of fibrosis in DD and test whether their inhibition provides resolution of fibrosis. Performing drug screens on surgical waste, patient-derived tissue, instead of e.g. rodent models, could be an effective and quick way of drug categorization for further experimentation or dismiss, and thus it could reduce the number of animal studies[35]. Future research will focus on understanding the role of the immune system in DD contracture and ways of altering the immune response to trigger matrix degradation. (Pre) clinical studies using the *ex vivo* culture method could potentially reveal immunological response triggered by candidate substances, and could be further used to delineate the exact mechanism of action of anti-fibrotic drugs.

Tumor Angiogenesis and Cancer

Cellular and extracellular context (microenvironment) has a great influence on tumor growth and development of drug resistance[47,48]. Tumor microenvironment consists of tumor cells, and abnormally functioning, non-malignant stromal cells such as fibroblasts, endothelial cells and infiltrating immune cells. Initial experiments with viral oncogene Src showed induction of transformation *in vitro* but not when overexpressed in embryos[49,50]. Moreover, mutated stromal fibroblasts have the potential to oncogenically transform normal epithelial cells[51]. Thus, the extracellular matrix and cellular context exert powerful influence on the behavior of cancer cells despite the presence of mutated oncogenic proteins. From this prospective, properties of the microenvironment and its impact on both normal and tumor cells is highly similar during fibrosis (as discussed previously) and cancer, which should be considered in the study of both human diseases. A hallmark in the progression of solid tumors is the formation of blood vessels (angiogenesis) within the primary tumor, which paradoxically requires the contribution of non-malignant cells[48]. Tumors perform neovascularization to sustain their growth utilizing non-malignant cells from their niche. In contrast to the wound healing angiogenesis where new vessels integrate within the existing network, in tumors there is destruction of the vasculature and formation of immature, leaky vessels. Several factors induce angiogenesis such vascular endothelial growth factor, platelet derived growth factor, hypoxia, BMPs along with matrix degradating enzymes that disrupt existing vasculature in order to recruit pericytes, endothelial and smooth muscle cells. In our studies we investigated the contribution of a BMP ligand, BMP9, in ALK1-mediated angiogenesis and tumor growth of human prostate cancer.

Sequestering of BMP9 by ALK1Fc ligand trap reduced tumor angiogenesis in primary prostate cancer xenografts and led to decreased tumor growth. Along with the anti-angiogenic effects, a direct effect of BMP9 inhibition on tumor cells was observed. Thus, BMP9 levels from the circulation, stroma and possibly secreted also from tumor cells, have a dual role in prostate cancer; pro-angiogenic effects on ALK1 expressing endothelial cells and pro-proliferative role on tumor cells via ALK2.

BMPs plays role in prostate tissue homeostasis as well as in tumor formation and metastasis to the bone, which is the predominant metastasis site for prostate cancer (PCa)[52]. Most BMP ligands and receptors are regulated by androgens and are expressed in the normal prostate tissue while up/downregulation is associated with primary or metastatic tumor[53]. BMP2, 4, 6, 7 and GDF15 have been extensively studied in this context and have tumor suppressing role on the proliferation of androgen-sensitive cancer cells[54]. Loss of function of many BMP ligands, SMAD4, SMAD8 and type II receptors (ACVR2A, ACVR2B, BMPRII) is associated with hormone insensitive stage of PCa and bone metastases[53-55]. BMP9 seems to have dual effects in PCa; proangiogenic mediated via ALK1 in endothelial cells and tumor promoting role in ALK2-expressing PCa cells, similar to pro-proliferative role on ovarian cancer[56] and hepatocellular carcinoma cells[57]. An explanation for the different PCa tumor effects between BMP9 and other BMP ligands, such as BMP7[58,59], may be the preference of BMP9 for ALK1 and ALK2 binding in different cell types, as opposed to BMP7 that utilizes ALK2, ALK3 and ALK6. Given the high affinity of BMPs to type I receptors this first binding step determines which type II receptor will be recruited to the complex causing different biological responses. Also, BMP9 binds to all three type II BMP receptors while BMP7 binds to BMPRII and ACVR2A[53]. Autocrine or paracrine BMP9 exert proliferative effect on PC3 tumor cells via ALK2 supported by microarray data indicating elevated ALK2 levels in PCa versus normal or benign prostate tissues (Chapter 6). Constitutively active ALK2 phosphorylates ENDOGLIN and promotes cell migration of PC3 cells[60]. Another potential mechanism is the synergy between BMPs and NOTCH signaling pathway; several studies prove that SMAD binding to transcription factors of NOTCH-intracellular domain class (NICDs) regulates myogenic differentiation and the balance between endothelial cell migration and quiescence[61]. In Chapter 6, we provide evidence of synergy between BMP9 and NOTCH ligand JAGGED that enhances the proliferation of PC3 cells and positively correlates with tumor progression in human microarray data.

Treatment with BMP9 ligand trap, ALK1Fc, showed promising anti-angiogenic and anti-tumorigenic effects in xenograft model of human PCa cells in mice (Chapter 6) and is currently being used in clinical trials for recurrent solid cancers (head and neck, ovarian, endometrial)[62]. ALK1 is an effective target for angiogenesis given its expression in endothelial cells and the complex formation with other pro-angiogenic factors such as BMP9, BMP10, TGFβ, ENDOGLIN[63-65]. However, the outcome on vessel quiescence or activation is often contradictory due to TGFβ-functioning via both ALK5 and ALK1 in endothelial cells leading to codependence or between SMAD1/5/8 and SMAD2/3 pathways[64]. Activation of both ALK1 and ALK5 by BMP9 and TGFβ, induce expression of proangiogenic factor VEGF[66]. BMP9/ALK1 signaling may inhibit and promote endothelial cell growth depending highly on the cell context[67]. ENDOGLIN, the type III auxiliary receptor of TGFβ signaling favours signaling via ALK1 by binding to BMP9, BMP10 as well as to TGFβ1, 3 and preventing their interaction with ALK5[67-69].

However, ENDOGLIN associates with other type I receptors such as ALK2, ALK3 and ALK6, thereby influencing TGFβ and BMP signaling in various ways[70]. Inhibitors of ENDOGLIN have been developed as anti-angiogenic treatment (TRC-105[71], ENDOGLIN-Fc[72])based on the ability of soluble ENDOGLIN to bind to BMP9 and BMP10 and inhibit vessel formation and tumor growth[73]. Several other anti-angiogenic drugs are investigated in clinical trials for cancer therapy[74]; thalidomide (ClinicalTrials.gov identifier; NCT00083551), AG-013736 (NCT00094107), Axitinib (NCT01321437), VEGF inhibitor SU5416 (NCT00006155), endostatin (NCT00518557).

Clinical Applications and Future Prospective

Deregulated TGFβ signaling is associated with disease progressio, for instance, chronic inflammation, cardiovascular disorders, organ fibrosis and malignancies, thus inhibition of this pathway is a promising therapeutic approach. Given the pleiotropic role of TGFβ signaling and its critical function in maintaining homeostasis of various tissues, therapies should tackle the pathogenic aspects of the signaling without interfering with homeostatic mechanisms.

As evidenced in Chapter 3, short term TGFβ inhibition may be beneficial in enhancing cell proliferation after acute liver injury; however, it may eventually delay or exacerbate the fibrogenic response needed for appropriate regeneration. Thus, from a translational perspective TGFβ inhibition must be spatiotemporally controlled in particular cell type(s), during specific stages of during disease progression. Such approach comes from liver fibrosis studies where decrease of collagen chaperone expression was performed *in vivo* using siRNA delivered via liposomes that were coated with retinol protein[75]. Based on the retinol-storing properties of collagen producing HSCs[11] the liposome-mediated drug delivery is beneficial in decreasing ECM protein secretion and limiting fibrosis[76]. Other studies performed HSC-specific drug targeting of ALK5 inhibitor LY-364947 utilizing the specific binding of mannose 6 phosphate human serum albumin (M6PHSA) on the insulin-like growth factor II receptor, which is expressed in HSCs[77].

A hypothetical therapeutic setting for Dupuytren's disease could be the administration of AONs (Chapter 4 and 5) prior to the surgical intervention or postoperatively, in order to counteract the TGFβ activity in the remaining MFBs. Early application of ALK5 AON could prevent destructive TGFβ action which is triggered by tissue damage during surgery, with consideration of the long term fibrotic effect that results from exposure to TGFβ[78]. Combination treatments targeting both the ECM and MFBs could be a promising approach; for instance treatment with matrix degrading enzymes, such as the FDA approved Clostridium Collagenase Xiaflex compound, would enhance the ability of TGFβ inhibitors (AONs or SMIs) to pass through the extracellular space and enter the MFBs. (Pre)clinical studies involving DD *ex vivo* culture method could potentially reveal anti-fibrotic candidate substances, that could be studied within a therapeutic context focused on patient-specific gene expression, drug and immune responses. Therefore, stratification of patients participating in clinical trials could be better monitored.

Systemic administration of TGFβ inhibitors in humans might have detrimental effects due to inhibition of the cytostatic and immune suppressive role of TGFβ in many organs leading

to formation of carcinomas and inflammation. However, studies in transgenic mice (soluble TGFβRII fused to Fc is under the control of mammary specific promoter MMTV-LTR) showed that lifelong exposure to TGFβRIIFc does not induce toxic effects or have negative impact on homeostasis[79] that is observed in mice with TGFβ gene deletion[80]. Most importantly, experimental induction of tumors in this mouse model showed that they are resistant to metastasis development[79]. A similar study showed beneficial effects in tumor apoptosis and migration in breast cancer after systemic administration of TGFβRIIFc, however no effect was observed on tumor angiogenesis[81]. Given the impact of the vasculature and surrounding stroma, combination treatment of TGFβ inhibitors with chemotherapeutics, immune modulatory agents or anti-angiogenesis factors[6] have gained scientific and clinical interest as an approach for cancer treatment[82-84]. Monotherapy in cancer treatment often results in drug resistance, while combinations of drugs that target tumor cell proliferation but also the microenvironment are more likely to slow progression to metastasis. A major limitation and growing concern in cancer field is multidrug resistance (MDR), a process via which tumor cells acquire mutations that provide them synchronous resistance to different drugs when given in combination[85]. To stop this vicious cycle of drug resistance and cancer progression to metastasis, several chemosensitising compounds have been developed to interfere with MDR, such as steroids, channel blockers or stealth liposomes[86]. Moreover, drug resistance to anti-angiogenic compounds is less prevalent because of the genome stability of endothelial cells compared to tumor cells[87].

One of the drawbacks of chemotherapy or radiation is the induction of apoptosis of normal cells which are not replenished but rather replaced by fibrotic scar tissue. In this case long term inhibition of TGFβ might be beneficial to block the progression of fibrosis in cancer survivors[88]. ECM deposition in the tumor stroma and MFB contraction affect the influx and outflux of fluid from the interstitial space causing high interstitial fluid pressure (IFP)[89]; a property found in tumors which hampers the delivery of drug compounds into the tumor cells[90]. This is another aspect where TGFβ inhibition in the tumor microenvironment may enhance the delivery and benefits of chemotherapy[91].

Collectively, the studies in this thesis aimed to improve insight on the molecular mechanisms exerted by TGFβ in various tissues and in different disease stages. Inhibition of the TGFβ shows pleiotropic effects consistent with the plethora of biological processes regulated by TGFβ. Modulation of the gene expression or protein activity of key components of the TGFβ/BMP pathway was performed using clinically releveant drug compounds. Our studies in homeostasis, fibrosis, tumor angiogenesis and cancer highlight the importance of a holistic view when interpreting the effects of TGFβ although therapeutic inhibitory approaches need to be cell type/tissue specific, administered at a specific stage of disease, in a controlled dosing scheme and taking into consideration the genetic variability of individual patients.

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