Pathogenesis and reversal of liver fibrosis: Effects of genes and environment
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chapter 6
closure:

summarizing discussion and conclusions

summaries

abbreviations
SUMMARIZING DISCUSSION

SUBJECT AND AIM
Fibrosis is a wound healing response to a variety of acute and/or chronic stimuli (ethanol, viral infection, drugs and toxins, cholestasis, and metabolic disease) (1, 2). It develops due to an increase in fibrillar collagen synthesis and deposition, along with insufficient remodeling (3, 4). This process is associated with a number of pathological and biochemical changes that lead to structural and metabolic abnormalities, and increased hepatic scarring (5, 6). The progression of liver fibrosis leads to cirrhosis, characterized by distortion of the normal architecture, formation of septae and nodule, altered blood flow, portal hypertension, HCC, and ultimately liver failure (7). Understanding the mechanisms and the pathways involved in the pathogenesis of the fibrogenic response and identification of potentially new therapeutic agents could provide novel therapeutic approaches for liver diseases in which fibrosis is a detrimental component.

The overarching aim of the studies described in this thesis, therefore, was to improve the understanding of the mechanisms underlying liver fibrosis, and to identify ways to slowdown the development and/or reduce liver fibrosis.

APPROACHES
To live up to the challenge, we used a battery of experimental models and approaches. In our in vitro studies, we used LX2 cells, a model system for activated HSCs, and primary MFs. In vivo, we studied Abcb4−/− mice, a model for chronic cholangiopathies that spontaneously develop liver fibrosis, as well as Abcb4−/− SMP8-Igf1+/−, a transgenic model created in one of the studies. These various models were challenged either by directly affecting the expression of genes of interest (IGFBP5, IGF1R, and IGF1) to scrutinize the potential roles of these gene products in the development of liver fibrosis. Protein expression levels were perturbed in vitro using adenoviral vector for overexpression, or siRNA for depletion. In vivo, we either used an AAV vector to deliver IGFBP5 to the hepatocytes of Abcb4−/− mice, or crossbred these mice with a transgenic strain to ensure IGF1-overexpression in activated HSCs. To test the hypothesis that nutrient deprivation benefits the fibrotic pathology, we studied the effects of fasting on existing liver fibrosis in Abcb4−/− mice. A broad range of molecular biology and biochemical techniques were employed, coupled with transcriptomics and system genetics approaches.

FINDINGS
Igf axis in vitro and in vivo
Our first effort to shed some light on the role of IGFBP5 in liver fibrosis has revealed its involvement in the survival of HSCs. Earlier studies have indicated cell- and tissue-type specific effects of IGFBP5. Its increased expression in fibrotic lung, skin and liver (8-11), in myogenesis, in vitro breast cancer cell growth, and in gingival epithelial cells (12-15), seems to be coupled with promotion of cell survival. Quite oppositely, in e.g. mammary gland involution IGFBP5 promotes
apoptosis of epithelial cells \textit{in vivo} and \textit{in vitro} (16-19). In liver fibrosis, apoptosis is reduced in activated HSCs that play a pivotal role in the initiation and perpetuation of pathology (20). Since IGFBP5 is spontaneously induced in HSCs cells upon activation (8), we hypothesized that it played a role in proliferation of HSCs in fibrotic liver. \textbf{Chapter 2} presents a study of the effects of IGFBP5 \textit{in vitro} in primary human MFs and LX2 cells, a model for partially activated human HSCs (21-23). In both cell lines an increased presence of IGFBP5, either by lentiviral overexpression or by addition of recombinant protein, promoted survival by lowering apoptosis. In support, silencing of endogenous IGFBP5 decreased the viability of LX2 cells, and of primary hepatic MFs, by an increase in apoptosis, pointing at IGFBP5 as a pro-survival factor in these two pro-fibrotic cell types.

The same study has addressed another important question raised whenever an IGF-binding factor is studied, namely what is the net effect of the interplay between IGF1 and IGFBP5? IGFBP5 binds IGF1 with high affinity and protects it from rapid degradation (24). Simultaneously, however, it prevents activation of the IGF1R and thereby inhibits prosurvival signaling by IGF1. Our \textit{in vitro} studies showed that, in contrast to IGFBP5 that acted via lowering apoptosis, IGF1 increased proliferation of LX2 cells. Moreover, we found no effect of IGF1 on IGFBP5 action, nor of IGFBP5 on IGF1 signaling, suggesting that in LX2 cells these two factors exert their effect via different, independent, routes. Several mechanisms may be involved in the IGF1 independent effect of IGFBP5. It was first shown \textit{in vitro} that IGFBP5 could increase activation of TGFβ1, a major pro-fibrotic cytokine, by plasmin via its interaction with tissue plasminogen activator (25). Consistently, mice overexpressing IGFBP5 had elevated tissue concentrations of plasmin (25). IGFBP5 directly activates tPA, which in turn activates plasmin. This further leads to activation of several MMPs involved in cellular migration/invasion, and in activation of TGFβ1 (25, 26). IGFBP-5 can also interact with TNFR1 (\textit{in vivo} and \textit{in vitro}) and block cell proliferation via inactivation of the NFκB signalling pathway (27). Furthermore, IGFBP5 interacts directly with several proteins from the matricellular family of proteins (osteopontin, thrombospondin-1 and tenascin-C) (28, 29), which are implicated in cellular adhesion, migration, wound healing or metastasis. In breast cancer cells IGFBP5 induces integrin-mediated Cdc42-dependent cell survival pathway, which leads to an adhesive, anti-m migratory, phenotype (involves stimulation of integrin-linked kinase (ILK), Akt and p53) and restricts epithelial–mesenchymal transgressions. As such, IGFBP5 might play role in limiting metastasis (30). IGFBP5 provokes formation of a filamin A (FLNa)-based nuclear shuttle that binds IGFBP5, recruits transcription factors, translocates to the nucleus and induces laminin (major component of the epithelial basement membrane involved in cell migration) gene transcription (31). In addition, IGFBP5 contains a nuclear localization sequence that mediates nuclear translocation of IGFBP5 to influence transcription of genes involved in osteoblast cell proliferation/differentiation (32). Evidence suggests that IGFBP5 binds and phosphorylates a putative IGFBP5 receptor on the osteoblast cell surface and stimulates downstream signaling pathways (33).

Much to our surprise, the following \textit{in vivo} study (presented in \textbf{Chapter 3}) revealed an antifibrotic effect of IGFBP5 in the livers of Abcb4\textsuperscript{-/-} mice. Our initial finding that IGFBP5 expression strongly increased during development of liver fibrosis in Abcb4\textsuperscript{-/-} mice (8), that IGFBP5 acts as a prosurvival signal \textit{in vitro} in LX2 cells, and two recent studies that reported a high expression of IGFBP5 in HCC and intrahepatic cholangiocarcinoma, suggested that it could have a profibrotic role \textit{in vivo}, and promote the survival of cancer cells and tumor formation (34, 35). The current study
has, however, shown a clearly reduced liver fibrosis in IGFBP5-treated animals, with considerably reduced portal bridging, and demonstrated the role of IGFBP5 in reduction of the existing scar tissue. To clarify the different role of IGFBP5 in liver and other organs, we analyzed its steady-state expression in the BxD genetic reference population (Chapter 3). The gene-phenotype correlations indicated an organ-specific regulation of IGFBP5, with a role that differed between organs but possibly protective in the liver. This could explain why IGFBP5 was found a profibrotic factor in lung and skin (10, 36), as opposed to its protective effect in liver fibrosis. A protective effect of IGFBP5 on hepatocytes in vivo (as shown by our research) is consistent with its prosurvival effect seen in activated HSC in vitro (37). Hence, even within the liver the prosurvival signal in stellate cells vs. hepatocytes may have counteractive effects. Apparently, in the in vivo setting of the Abcb4−/− mouse the protective effect on hepatocytes prevails over the increased survival of stellate cells.

The most likely mechanism of the anti-fibrotic effect of IGFBP5 expression in the Abcb4−/− mice is reduction of portal inflammation, which is initially caused by leakage of toxic bile, one of the most prominent pathological features in this model for chronic cholangiopathy (38). Overexpression of IGFBP5 lowers the influx of inflammatory cells into the area, lowering the production of inflammatory cytokines, known to stimulate collagen synthesis and to enhance hepatocyte damage. Fewer damaged hepatocytes and fewer activated inflammatory cells means less oxidative stress (39, 40) that enhances the expression of profibrotic genes in human HSCs and MFs (41, 42), which may additionally alleviate liver fibrosis.

IGFBP5, however, also may have a more direct effect. The increased amount of p21 in IGFBP5-overexpressing livers may be instrumental for the reduced proliferation. p21 serves as a negative cell cycle regulator under stress conditions caused by various factors (growth factor deficiency, DNA damage, and exposure to heavy metals or antiproliferative cytokines, particularly TGFβ). Upon DNA damage, p21 blocks the transition from G1 into S phase or from G2 into mitosis, enabling the repair of damaged DNA (43). Furthermore it regulates PCNA that binds to damaged DNA (44) reducing its nuclear presence as seen in our study. In the cytoplasm, p21 protein has an anti-apoptotic effect through binding to and inhibiting caspase 3, as well as the apoptotic kinases ASK1 and JNK (45). p21 can act as a tumor suppressor, in particular by participating in the launch of a senescence program (replicative senescence as well as stress-induced premature senescence) (46). More and more evidence points to IGFBP5 as an important player in cellular senescence, process that allows an escape from uncontrolled cell proliferation, thereby halting tumorigenesis (47, 48). Regardless the cause of senescence, two tumor suppressor pathways, the p53-p21 and p16-pRB, are typically responsible for the following growth arrest and senescence (48). Also several cytokines, including IFNα, IFNγ, TGFβ, IGFBP3, IGFBP5 have been reported to regulate senescence (49–52). Increased IGFBP5 expression is associated with senescence in vitro in pulmonary fibrosis, human dermal fibroblasts and endothelial cells (53-57). Knockdown of IGFBP5 partially reverses senescence in aged human umbilical-vein endothelial cells (HUVEC), whereas IGFBP5 induces and accelerates premature senescence in young HUVEC cells. IGFBP5-induced senescence is associated with induction of the tumour suppressor p53 (51).

Following the path in the IGF-axis upstream of IGFBP5, in Chapter 4 we tested the influence IGF1 on liver fibrosis in a model for chronic cholangiopaties. It has been shown by two independent studies, that increased expression of IGF1 significantly reduced liver fibrosis in CCl4
treated rats (58, 59). In a follow-up phase I-II clinical trial, IGF1 administration improved liver function in patients suffering from liver cirrhosis (60). The potential use of IGF1 in chronic cholangiopathies, however, has not been studied. Since the biliary epithelium expresses IGF1R (61), and IGF1 protects cholangiocytes against cholestatic injury in vitro (62), we postulated that it could be beneficial in chronic cholangiopathy. To study it, we crossbred the Abcb4−/− mice with a transgenic mouse that overexpresses IGF1 in activated HSCs. This cross showed increased expression of profibrotic factors (Tgfβ and Pdgf), an increased hydroxyproline level (2-fold higher than previously reported (63)), as well as an enhanced deposition of ECM. IGF1 overexpression also increased the expression of Timp1, which could reduce MMP activity and ECM degradation in our model.

These observations all point to a more prominent fibrosis in Abcb4−/− in the presence of IGF1. The increased serum ALP and bilirubin levels after 12 weeks of cholate diet in the current study indicate that IGF1 could increase proliferation and survival of cholangiocytes, and independently cause cholestasis in hepatocytes. Though these responses are aimed at repair of the bile duct epithelium, increase of IGF1 above the normal levels induces extensive proliferation resulting in bile duct abnormalities. Although unexpected, the different effect of IGF1 overexpression between the previously studied CCl4 model (58, 59) and this model for chronic cholangiopathy is most likely due to the difference in severity of the models. To clarify this, we have performed bile duct ligation (BDL) in the SMP8-IGF1. The data revealed an absence of protective effect of overexpression of IGF1 also in BDL model. In this model, however, no difference in bile duct proliferation was seen between the WT and the SMP8-IGF1 mice.

A difference in pathogenesis between the models may explain the discrepancy between Abcb4−/− mice and BDL-induced mouse models of liver fibrosis. Ligation of the common bile duct results in an acute interstitial (biliary) fibrosis, associated with massive proliferation of bile ducts, (only rarely observed in man). On the other hand, in Abcb4−/− mice progression of fibrosis is spontaneous due to cholangiocyte proliferation and massive up-regulation of profibrogenic genes, and bears more resemblance to human biliary fibrosis (63). The damage in the Abcb4−/− model is chronic and less severe and localized to the portal areas (64), which results in a persistent increase in IGF1 signaling enhancing cholangiocyte proliferation (61). In PBC patients suffering from chronic bile duct damage, activation of the IGF1 system plays a role in the survival of cholangiocytes (65). In addition, IGF1 enhances the proliferation of liver cystic epithelium and reduction of its expression reduces the size of liver cysts in a mouse model (66). This may explain the formation of extremely large, but ill-developed bile-duct structures observed in that model, leading to the formation of strictures. Presence of strictures (though not specifically demonstrated) could explain the cholestasis that, according to serum bilirubin and ALP level, has eventually developed in our transgenic mice.

**Fasting in abcb4−/− mice**

Driven by the data from human and rodent studies (67, 68), in which fibrotic profiles in obese individuals were improved by weight loss, we postulated that the adaptation to nutrient deprivation would also be beneficial for fibrosis caused by chronic cholangiopathy. The fasting study in Abcb4−/− mice (Chapter 5) has indeed exposed a surprisingly quick improvement in pathology of fibrotic liver - already after 12h of food deprivation. Starvation provokes a massive response of the body to
preventing an irreversible loss of resources (69). In mice, within hours after the last meal, the organs respond with changes in gene expression mainly in general metabolism (70). The role of the liver is to provide energy for glucose-dependent tissues, by glycogenolysis, gluconeogenesis, ketogenesis, and fatty-acid β-oxidation (71). The basic architecture of the lobules and the zonation are not affected, but the cell size declines in prolonged fasting, when murine liver restores partly its glycogen deposits, and much of gene expression returns to control values (72). In Abcb4-/ mice, collagens, fibronectin and vimentin, responsible for the structural integrity of the ECM, were strongly affected by fasting. The downregulation of collagen mass in fasted fibrotic livers occurred in early stages of starvation, before the other energy sources were depleted, pointing to an active ECM remodeling, rather than to an ad hoc degradation for mere provision of amino acids. As a part of tissue remodeling, fibrotic livers of Abcb4-/ mice responded to caloric restriction by a decrease in their pathologically high proliferation. Liver fibrosis was further attenuated by reduced number of activated HSCs, most likely driven by downregulation of TGFβ1 (73). The impressive decrease in inflammation in fasted Abcb4-/ mice, and migration of macrophages from the periportal tract through the acinar zone 2 towards the central vein, seem a likely driving force for improved pathology. This change in number and localization of resident and infiltrated macrophages may be causal in resolution of the parenchymal fibrosis (by altered production of MMPs (74, 75)).

A caveat of the studies presented in this thesis could be seen in the fact that liver fibrosis was addressed mainly in a single in vivo model (of chronic cholangiopathy). Clearly, the effects of IGFBP5 should be further tested in in additional animal models including a viral administration of IGFBP5 to the newly established IGF1 overexpressing model of Abcb4-/ mice. In addition, apart from hepatocytes, other cell types involved in fibrosis should be specifically targeted in Abcb4-/ mice, using different specific AAV vectors (HSCs by AAV5. Similarly, the effect of fasting should be studied upon subjecting animals to short(er) and/or alternate day fasting in more than one fibrotic model. The mechanism behind the antifibrotic effects seen in this thesis should be further elucidated.

CONCLUSIONS

In a nutshell, the studies presented in this thesis have shown that progression of liver fibrosis in Abcb4-/ mouse model of chronic cholangiopathies can be halted and even reversed by targeted gene transfer of IGFBP5, or by starvation.

We have demonstrated different, cell-type specific effects of IGFBP5. Firstly by overexpressing it in vitro, in activated HSCs and MFs, where it improved survival by reducing apoptosis (in an IGF1-independent manner), suggesting its possible profibrotic role. However, prolonged expression of IGFBP5 in vivo, in hepatocytes of Abcb4-/ mice, decreased their proliferation, reduced inflammation and oxidative stress, and lowered number of activated HSCs and ECM deposition, thereby evidently reducing liver fibrosis.

The star of the IGF-axis, IGF1, did not live up to the expectations in the Abcb4-/ model. In a new transgenic mouse model created in our study, increased expression of IGF1 in Abcb4-/ mice aggravated the pathology by increasing cholangiocyte proliferation, opposite to the anti-fibrotic action of IGF1 in acute parenchymal liver damage caused by BDL or CCl4. In contrast to drug-
induced liver fibrosis, IGF1 administration therefore does not seem an option for treating fibrosis caused by chronic cholangiopathies.

Furthermore, fasting appeared a strong metabolic prosurvival stimulus that should not be underestimated. We showed that as short as 12 hours of food deprivation positively influenced pathology in fibrotic Abcb4−/− mouse liver, by decreasing inflammation, and by actively increasing matrix remodeling.

These studies were the first to tackle the problem of liver fibrosis by specifically targeting IGFBP5, and by introducing fasting challenge. Though we have not eliminated the cause of fibrosis by any of the approaches (i.e. bile composition remained unchanged), we did alleviate its consequences. This may have opened a door for new therapies of liver fibrosis.

References


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SUMMARY

As a normal wound-healing response to liver injury, fibrosis is reversible - normal architecture is restored by fibrolysis, and ECM producing cells are removed by apoptosis. However, as reviewed in Chapter 1, chronic liver injury increases production of fibrogenic signals by resident and infiltrating liver cells, causing an imbalance between fibrogenesis and fibrolysis, scar formation, architectural distortion, cirrhosis, and eventually liver failure. With liver transplantation as the only effective treatment, the lack of donors and surgical contraindications impel for treatments to halt the progression of disease.

HSCs, mesenchymal cells vital to hepatic function and its response to injury, play a pivotal role in the development of liver fibrosis. IGFBP5 was shown to be highly expressed in fibrotic livers of Abcb4−/− mice. Therefore, in Chapter 2, we examined the influence of IGFBP5 on HSCs and MFs in vitro. Using gain- and loss-of-function approaches (overexpression by lentiviral transduction, or silencing by siRNA), we showed that IGFBP5 influenced the survival of human LX2 cells, a model for (partially) activated HSCs, and of hepatic MFs. Their endurance was improved without enhancing proliferation, by lowering the level of apoptosis, via an IGF1-independent mechanism. Moreover, IGFBP5 increased the expression of genes involved in ECM deposition.

The finding that IGFBP5 promotes survival of HSCs and MFs in vitro has led us to investigate its role in vivo, in Abcb4−/− mice. These mice spontaneously progress to severe biliary fibrosis, due to absence of biliary phospholipids that leads to primary sclerosing cholangitis. Abcb4−/− mice are also a model for human MDR3 deficiency, ranging from progressive familial intrahepatic cholestasis type 3 to adult liver cirrhosis, which makes them an attractive model for testing potential antifibrotics. In Chapter 3 we demonstrate that prolonged liver-specific overexpression of IGFBP5 alleviated the hepatocyte damage, as demonstrated by improved biomarkers of liver injury, and decreased their proliferation, possibly by arresting cell cycle, accompanied by senescence. Furthermore, overexpression of IGFBP5 reduced inflammation, indicated by decreased presence of markers for infiltrating and resident macrophages, neutrophils and monocytes. Consequential lowered release of proinflammatory cytokines may explain the decreased oxidative stress in these livers. The resulting reduced presence of activated HSC/MFs and reduced expression of collagens led to a decreased amount of ECM, ameliorating pathology in the model for chronic cholangiopathy.

A recent study has indicated that biliary epithelium expresses IGF1R, and that IGF1 protects cholangiocytes against cholestatic injury in vitro. To establish the effect of IGF1 on the existing cholestatic injury in vivo, we subjected the Abcb4−/− mice to a prolonged increase in hepatic IGF1 expression, by creating a transgenic animal. Chapter 4 shows that sustained overexpression of IGF1 in fibrotic livers increased cholangiocyte proliferation, enhanced inflammation and reduced matrix remodeling, bringing about an increase in deposition of scar tissue and progression of liver fibrosis. IGF1 administration therefore does not seem an option for treating fibrosis caused by chronic cholangiopathies.

The research presented in Chapters 2-4 has challenged the Abcb4−/− mice directly, by affecting the expression of genes of interest (IGFBP5 and IGF1), to scrutinize their potential roles in the development of liver fibrosis. Along the same line, we studied how nutrient deprivation could...
affect liver fibrosis in the same model. In Chapter 5 we show that food deprivation causes a rapid adaptive response in Abcb4<sup>-/-</sup> mice, already after 12h. A striking decrease in inflammation in fasted Abcb4<sup>-/-</sup> mice seems a likely driving force for a cascade of events, including decreased hepatocyte proliferation, lowered number of activated HSCs/MFs, decreased production of ECM components, and increased expression of genes involved in tissue remodeling.

The studies described in this thesis embarked upon the problem of biliary fibrosis by delineating the roles of specific players in IGF-axis, and by introducing a fasting challenge. Though we have not eliminated the cause of fibrosis by any of the approaches (i.e. bile composition remained unchanged), we did alleviate the consequences, which leaves the door to new therapies for liver fibrosis slightly ajar.
Doordat schade aan de lever kan leiden tot littekenvorming in de lever, noemen we dit proces fibrose. De strengen littekenweefsel in de lever bestaan voor een groot deel uit bindweefsel zoals collageen. Leverfibrose kan beschouwd worden als een ontstekings of wondheilingsproces en hoeft daarom niet noodzakelijkerwijs tot permanente leverschade te leiden. Collageen bevattende fibrotische littekens kunnen ook weer opgeruimd worden.

Wanneer er sprake is van constante leverschade gaat er iets mis en wordt de fibrotische littekenvorming versterkt. In Hoofdstuk 1 wordt beschreven dat door chronische leverschade de afbraak van collageen niet goed meer verloopt. Hierdoor gaat het proces van littekenvorming verder waardoor de leverstructuur verwoest wordt, met als gevolg cirrose en uiteindelijk een complete teloorgang van de lever.

Het is al lang bekend dat stellaatcellen cruciaal zijn voor het proces van lever fibrose. Omdat wij vonden dat in fibrotische muizenlevers het IGFBP5 eiwit sterker werd aangemaakt hebben we in Hoofdstuk 2 onderzocht wat het effect van IGFBP5 op stellaatcellen is. We konden aantonen dat stellaatcellen gestabiliseerd worden door IGFBP5 zonder dat ze sneller gingen delen. Belangrijk is ook de vinding dat stellaatcellen door IGFBP5 meer collageen gaan produceren.

Deze experimenten zijn allemaal gedaan met stellaatcellen in celkweek, de volgende stap was het onderzoeken van het effect van IGFBP5 in een dierrmode van leverfibrose. In Hoofdstuk 3 laten we zien dat de schade aan de lever van muizen met fibrose verminderd kan worden door de productie van IGFBP5 in de levers te verhogen. In tegenstelling tot onze vindingen in stellaatcellen in celkweek in Hoofdstuk 2, blijkt in muizen het IGFBP5 eiwit juist minder ontsteking en collageenvorming in fibrotische levers te veroorzaken.

In de wetenschappelijk literatuur wordt gesuggereerd dat het IGF1 eiwit een andere belangrijke factor is die de lever tegen schade door fibrose zou kunnen beschermen. In Hoofdstuk 4 beschrijven we daarom het gevolg van het verhogen van de hoeveelheid IGF1 eiwit in de lever van muizen met fibrose. Tot onze verassing bleek door IGF1 de fibrose juist erger te worden, er was meer litteken- en collageenvorming in de lever. Het toedienen van IGF1 is dus geen oplossing voor het probleem van leverfibrose.

In Hoofdstukken 2-4 hebben we leverfibrose in modelsystemen, cellen en muizen, bestudeerd en proberen te behandelen door de eiwitten IGFBP5 en IGF1. In Hoofdstuk 5 hebben we een andere weg behandeld door muizen met leverfibrose te laten vasten. We vonden hierbij dat het verminderen van de voedselinname in muizen met leverfibrose een snelle vermindering van de leverontsteking tot gevolg had. Al 12 uur na het begin van het dieet konden we aantonen dat er minder collageen in de lever werd gevormd.

In dit proefschrift hebben we laten zien dat IGFBP5 leverfibrose vermindert en dat IGF1 leverfibrose juist verergert. We hebben ook gevonden dat vasten leverfibrose kan verminderen. Alhoewel we met deze vindingen de oorzaak van leverfibrose niet aan hebben kunnen pakken, leven onze resultaten toch goede aanknopingspunten op waarmee wellicht nieuwe geneesmiddelen voor fibrose gevonden kunnen worden.
Fibroza kao normalan odgovor na povredu tkiva, je u osnovi reverzibilan proces. Normalna arhitektura jetre se obnavlja razgradnjom fibroznog matriksa (ožiljačnog tkiva) u procesu zvanom fibroliza, dok ćelije koje ga produkuju bivaju odstranjene putem apoptoze. Hronično oštećenje jetre, medijutim, kako je elaborirano u Poglavlju 1, dovodi iznova i iznova do aktiviranja fibrogenih signala, kako od strane oštećenih parenhimskih ćelija jetre (hepatocita), tako i od strane lokalnih i infiltrirajućih ćelija imunog odgovora, imajući za posledicu narušavanje normalne arhitekture, ciroza i, na kraju, prestanka rada jetre. Transplatacija jetre, koja je još uvek jedini efikasni tretman, je suočena sa teško premostivim izazovima, pre svega manjkom donora, a potom i kontraindikacijama koje prate sam hiruški zahvat, što nameće potrebu za hitnim razvojem tretmana koji bi zaustavili napredovanje same bolesti.

Poznato je da stelatne ćelije, vitalne za normalno funkcionisanje jetre i njen odgovor na oštećenje/povredu, imaju vodeću ulogu u razvoju fibroze. Naša prethodna istraživanja su pokazala da je proizvodnja IGFBP5 proteina značajno povišena u fibrozi kod miševa sa holestazom jetre. Stoga je istraživanje prikazano u Poglavlju 2 imalo za cilj da prouči specifičan uticaj IGFBP5 na stelatne ćelije i miofibroblaste. IGFBP5, ispostavilo se, poboljšava njihovo preživljavanje smanjenjem nivoa apoptoze, ne utičući na ćelijsku deobu. Pokazalo se i da IGFBP5 povećava prisustvo gena uključenih u proizvodnju proteina ožiljačnog tkiva, ukazujući na njegovu moguću profibrotičku ulogu.

Ovi rezultati iz eksperimenata u ćelijkoj kulturi su bili uvod u sledeći korak – da se prouči uloga IGFBP5 u životinjskom modelu fibroze jetre. Korišćeni miševi se karakterišu potpunim nedostatkom fosfolipida u žuči, što pojačava njihova detergentska svojstva, čini je toksičnom i dovodi do spontanog razvoja fibroze. U Poglavlju 3 smo pokazali da povišeno prisustvo IGFBP5 u jetri, dovodi do smanjenja oštećenja hepatocita, koje za posledicu ima smirivanje upalnih procesa i, shodno tome, smanjen oksidativni stres. Kao posledica, došlo je do smanjenja broja aktiviranih stelatnih ćelija i miofibroblasta, smanjenja količine ekstracelularnog matriksa, što je na kraju dovelo do neočekivanog ublažavanja patologije, sugerišući da IGFBP5 u jetri, za razliku od izolovanih stelatnih ćelija, ima antifibrotičku ulogu.

Odnedavno je poznato da epitelijalne ćelije u žučnim kanalima (holangiocite) imaju i receptor za IGF1 protein i da ih, u ćelijkoj kulturi, IGF1 štiti od oštećenja izazvanoj toksičnom žuči. Da bi smo ustanovili uticaj IGFBP5 na nepristojnost oštećenja u jetri, proizveli smo transgenog miša, sa povišenom količinom ovog proteina u fibrotičkoj jetri. U Poglavlju 4 smo pokazali da stalna povišena proizvodnja IGF1 u ovim miševima stimulise deobu holangiocita, što je na kraju dovelo do neuronodirano uključivanje patologije, sugerišući da IGF1 u jetri, za razliku od izolovanih stelatnih ćelija, ima antifibrotičku ulogu.

U studijama opisanim u Poglavljima 2 - 4 direktno smo uticali na ekspresiju gena od interesa (IGFBP5 i IGF1), da bi proučili njihovu moguću ulogu u razvoju fibroze. Paralelno, proučavali smo i
kako smanjeno unošenje hrane utiče na fibrozu u istom modelu. Rezultati prikazani u Poglavlju 5 ukazuju na vrlo brz adaptivni odgovor na gladovanje. Upadljivo smirivanje zapaljenjskih procesa u jetrama izgladnjvanih životinja već posle 12 sati je, čini se, zamajac za kaskadu događaja koja uključuje smanjenje brze deobe hepatocita, inače karakteristične za ove miševe, smanjenje broja aktiviranih stelatih ćelija i miofibroblasta, kao i smanjeno skladištenje komponenata ekstracelularnog matriksa, i povišenu ekspresiju gena uključenih u remodelovanje tkiva.

Istraživanja prikazana u ovoj tezi su, dakle, bila pokušaj da se rasvetli problem bilijarne fibroze – bilo određujući uloge pojedinih gena, bilo uvodeći gladovanje kao izazov za fibrotičnu jetru. Iako direktan uzrok fibroze nije eliminisan ni jednim od pristupa (tj. sastav žuči ostao je nepromenjen), posledice fibroze su ublažene, što ostavlja odškrinuta vrata za razvoj novih terapija.
ABBREVIATIONS

AAV  adeno-associated virus
ABCB4 ATP-binding cassette, sub-family B member 4
ALT  alanine aminotransferase
BCL2  B-cell CLL/lymphoma 2
BDL  bile duct ligation
BrdU  bromo-2’-deoxy-uridine
CB1  endocannabinoid receptor
CC  cholangiocarcinoma
CCl4  carbon tetrachloride
CD95  cell surface death receptors
CLD  chronic liver diseases
ECM  extracellular matrix
EGF  epidermal growth factor
F4/80  EGF-like module-containing mucin-like, hormone receptor-like 1
FCS  fetal calf serum
FGF2  fibroblast growth factors 2
GFP  green fluorescent protein
GSH  glutathione
GSSG  glutathione disulfide
HCC  hepatocellular carcinoma
HGF  hepatocyte growth factor
HSCs  hepatic stellate cells
IFNα  interferon alpha
IFNγ  interferon gamma
IGF1  insulin-like growth factor 1
IGF1R  insulin-like growth factor 1 receptor
IGFBP5  insulin-like growth factor binding protein 5
ILK  integrin-linked kinase
JNKs  c-Jun N-terminal kinases
Mac1 integrin alpha M
MFs myofibroblasts
MMPs matrix metalloproteinases
MPO myeloperoxidase
NAFLD non-alcoholic fatty liver disease
NASH non-alcoholic steatohepatitis
NFκB nuclear factor κB
NGF nerve growth factor
p21 cyclin-dependent kinase inhibitor 1A
p53 tumor protein 53
PCNA proliferating cell nuclear antigen
PDGF platelet-derived growth factor
PDGFβ-R platelet derived growth factor receptor β
PI3 phosphatidylinositol 3-kinases
rIGF1 recombinant insulin-like growth factor 1
rIGFBP5 human recombinant insulin-like growth factor binding protein 5
ROS reactive oxygen species
SAβ-gal senescence-associated β-galactosidase
siRNA small interfering RNA
TGFα transforming growth factor α
TGFβ1 transforming growth factor, beta 1
TIMP1 tissue inhibitor of metalloproteinase 1
TNFα tumor necrosis factor alpha
uPA urokinase-type plasminogen activator
VEGF vascular endothelial cell growth factor
α-SMA actin, alpha 2, smooth muscle