SUPPLEMENTAL MATERIALS AND METHODS

Reagents

All general chemicals were purchased from Carl Roth GmbH (Karlsruhe, Germany) or from Sigma-Aldrich Co. (USA), unless otherwise stated, and were of the highest quality.

Cell culture

The murine HSC cell line JS1,[1], freshly isolated primary hepatic stellate cells, (isolation method see below), the murine cholangiocyte cell line 603B and human MMNK-1 cells were cultured in Dulbecco's modified Eagle's medium (Lonza, Basel) with 10% fetal bovine serum, 1% penicillin (100 IU/ml)/streptomycin (100 µg/ml), and 2 mM glutamine. The cells were maintained in a 37°C humidified atmosphere containing 5% CO2.
24 hours prior to treatment with 10 ng/ml TGF-β2 (PeproTech GmbH, Hamburg, Germany), medium was changed to 'starvation medium' with 0.5% FBS.

Animal models

Animals were housed under specific-pathogen–free conditions in the animal facility of the Medical Faculty Mannheim, Heidelberg University, received humane care and water and food ad libitum. All animal protocols were in full compliance with the guidelines for animal care and approved by the government's Animal Care Committee (35-9185.81/G-138/13, Regierungspräsidium Karlsruhe).

Mouse models of non-biliary liver diseases

PHx and CCl_4 treatment for acute liver damage was performed as described [2]. To induce liver fibrosis by chronic CCl_4-treatment of C57BL6/N mice were exposed to 1.6g/kg CCl_4 dissolved in olive oil (twice/week) as described [3]. STAM mouse (background C57BL6/j) livers were purchased from Stelic Institute and Co., Inc. (Japan),[4].

These models were used for data presented in supplementary figure 1.

Human Patient samples

As PSC and PBC are rare diseases, we were restricted to relatively small sample cohorts. Four distinct PSC/PBC patient cohorts were used for the analysis: 1. The Regensburg cohort including tissue and RNA of 10 PSC and 3 PBC patients and 5 control livers. Liver tissue specimens were collected from patients who underwent liver transplantation (n=7) or liver resection (hemihepatectomy, n=6). Normal (control) liver tissues were obtained from liver resections of hepatic metastases of extrahepatic tumors and with no microscopic changes of liver disease identified by a pathologist. Tissue samples and annotated data were obtained and experimental procedures were performed within the framework of the non-profit foundation Human Tissue and Cell Research (HTCR, Regensburg, Germany) 2. Expression and clinical data from 17 patients in part published as GSE79850. 3. PSC/PBC patient cohort with 25 patients´ and 38 control liver tissues published as GSE61260. 4. The Polish cohort includes cirrhotic liver samples of patients with PBC (n=10) and PSC (n=10) who underwent liver transplantation. Control liver tissues (n=5) were acquired from large margin liver resections of patients with colorectal metastases with no microscopic signs of liver disease as estimated by a pathologist, all collected in the Department of General, Transplant and Liver Surgery, Medical University of Warsaw, ,[5]. Additionally, a patient cohort GSE46960 comprising samples from biliary atresia patients (n = 64) was analyzed. Normal samples (n = 7) are liver biopsies from deceased-donor children. Additionally, 14 samples from patients with liver disease associated with intrahepatic cholestasis are included.

Cohorts of non-biliary derived liver disease patients

The following cohorts of patients with liver diseases of non-biliary associated etiologies were analyzed: E-GEOD-59045, E-GEOD-48452, E-GEOD-61256, E-MTAB-4856. In GSE31308 and E-GEDO-28619.

Plasma analysis for liver function parameters

Whole blood was obtained by retrobulbar blood draw in mice and collected in Lithium-Heparin microvettes. After centrifugation, plasma was transferred to an Eppendorf tube and either stored at −80°C or used for immediate analysis. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (AP), glucose, cholesterol, triglycerides, proteins, bilirubin and glutamate dehydrogenase (GLDH) plasma contents were measured with the Cobas® 8000 analyzer (Roche, Mannheim, Germany).

Determination of *TgfB2*-specific antisense oligonucleotide biodistribution (ISTH0047, AON) in livers of wild type and MDR2-KO mice

TgfB2-specific antisense oligonucleotide (ISTH0047, AON hereafter) distribution, was determined in 16-week-old male and female Balb/c wildtype and MDR2-KO mice. Balb/c mice were injected subcutaneously (s.c.) with a single dose of 10 mg/kg body weight FAM-labelled AON. Cell-type specific distribution of FAM-labelled AON in liver tissue of Balb/c mice was determined by confocal microscopy. Cell type-resolved AON biodistribution was further determined in Balb/c and MDR2-KO mice. Balb/c and MDR2-KO mice were injected s.c. with a single dose of 10 mg/kg body weight digoxigenin-labelled AON. All mice were sacrificed 72h after AON administration and livers samples were immediately snap-frozen in liquid nitrogen or paraffin-embedded according to standard operation procedures. Specimen sampling was performed for immunofluorescence analyses and immunohistochemistry.

Simultaneous isolation of four liver cell types based on magnetic bead separation

Primary liver cells were isolated from Balb/c wild-type and MDR2-KO mice by collagenase perfusion followed by liver tissue separation with the autoMACS® Pro Separator (Milteny) and cell-type-specific magnetic beads (Microbeads, Milteny). Mice were anesthetized with

an intraperitoneal injection of a Ketamine/ Xylazine hydrochloride mixture and livers perfused by a flow-through with EGTA solution and then Collagenase D solution. Removed livers were placed in the autoMACS® Pro Separator (Milteny) for tissue dissociation. Hepatocyte separation was done using low-speed centrifugation and purification by Percoll (VWR). The single-cell suspension was labeled with cell-type-specific MicroBeads for ~15-30 min, transferred to a MACS Column and placed in the MACS Separator which induced a magnetic field and created a high gradient in the column. While positive-labeled cells were retained in the magnetic field, unlabeled cells passed through the column (negative fraction). These steps were repeated using the respective negative fraction sequentially for all different cell-types with specific magnetic beads from Milteny (for Kupfer cells CD11b, for LSEC CD146, for stellate cells CD271). Cell viability was determined by counting with Trypan blue and purity was assessed either by immunofluorescence or qPCR.

RNA isolation and cDNA synthesis

Total RNA was extracted according to the manufacturer's instructions using InviTrap Spin Universal RNA Mini Kit, Berlin, Germany) or with Trizol reagent (LifeTechnologies) and were purified via chloroform extraction. The RNA concentration was determined with the Tecan infinite M200 microplate reader (Tecan, Switzerland) and subsequently reversely transcribed with RevertAid H Minus M-MuLV Reverse Transcriptase (200 u/µl) to synthesize cDNA from from 0.5 -1 µg total RNA according to the manufacturer's protocol.

Quantitative real-time PCR

Real-time polymerase chain reaction (qPCR) was carried out with Power SYBR Green (Life Technologies) using the Stratagene MX 3005 P system or Abiprism StepOne Plus system. The primers and probes are listed below. A dissociation curve was analyzed to guarantee primer specificity. Only primers with a unique dissociation peak were selected. To compensate for the variation between qPCR runs, the target gene expression was normalized to the expression of the endogenous, unregulated reference gene PPIA. The relative quantity of target genes was determined according to the ΔΔCt method,[6].

The following probes and reagents for Taqman (Thermoscientific) RT-PCR analysis of *TgfB2* expression in STAM mouse samples was used: Mm03024009_m1, (*TgfB2*), Mm02342429_g1 (*Ppia*) as housekeeping gene, TaqMan® Universal Master Mix II.

qPCR primers used in combination with SybrGreen

Gene name	Forward	Reverse	species
Ppia	GAGCTGTTTGCAGACAAAGTC	CCCTGGCACATGAATCCTGG	mouse
TgfB1	AGGGCTACCATGCCAACTTC	CCACGTAGTAGACGATGGC	mouse
TgfB2	GCAGATCCTGAGCAAGCTG	GTAGGGTCTGTAGAAAGTGG	mouse
Col1a1	ACGTGGAAACCCGAGGTATG	TTGGGTCCCTCGACTCCTAC	mouse
Col3a1	ACGTAAGCACTGGTGGACAG	GGAGGGCCATAGCTGAACTG	mouse
Acta2	TTCGCTGTCTACCTTCCAGC	GAGGCGCTGATCCACAAAAC	mouse
PparG	TCCAGCATTTCTGCTCCACA	ACAGACTCGGCACTCAATGG	mouse
TnfA	CCCTCACACTCACAAACCAC	ATAGCAAATCGGCTGACGGT	mouse
Timp1	CGAGACCACCTTATACCAGCG	ATGACTGGGGTGTAGGCGTA	mouse
Il6	TAGTCCTTCCTACCCCAATTTCC	TTGGTCCTTAGCCACTCCTTC	mouse
Il1B	CCCAACTGGTACATCAGCACCTC	GACACGGATTCCATGGTGAAGTC	mouse
Ctgf	AGATTGGAGTGTGCACTGCCAAAG	TCCAGGCAAGTGCATTGGTATTTG	mouse
Muc1	CCAAGCGTAGCCCCTATGAG	GTGGGGTGACTTGCTCCTAC	mouse
Fibronectin	ATACCGTTGTCCCAGCTGTC	AGCTCTGCAACGTCCTCTTC	mouse
PdgfBr	GTTGTACCTTCCGCAGAGAATG	GTCACCCAAGGTACGGTTGT	mouse
ErrG	TCAAAGCCCTCACCACACTCT	TCCTGCTCAACCCCTAGTAGATTC	mouse
rS18	AAACGGCTACCACATCCAAG	CCTCCAATGGATCCTCGTTA	human
HPRT	CCTGGCGTCGTGATTAGTGA	CGAGCAAGACGTTCAGTCCT	human
TGFB1	TGGTGGAAACCCACAACGAA	GAGCAACACGGGTTCAGGA	human
TGFB2	GCAGATCCTGAGCAAGCTG	GTAGGGTCTGTAGAAAGTGG	human
CD45	GATGCCTACCTTAATGCCTCTG	TCCACATTCCACATTCTCATTAAC	human
PPARG	GTGGCCGCAGAAATGACC	CCACGGAGCTGATCCCAA	human
FIBRONECTIN	ACAAGCATGTCTCTCTGCCAA	TCAGGAAACTCCCAGGGTGA	human
PDGFBR	CCCTTATCATCCTCATCATGC	CCTTCCATCGGATCTCGTAA	human
ACTA2	AAGAGCATCCGACACTGCTGAC	AGCACAGCCTGAATAGCCACATAC	human
COL1A1	CGGACGACCTGGTGAGAGA	CATTGTGTCCCCTAATGCCTT	human
TIMP1	GCACTCATTGCTTGTGGACG	CGGGCAGGATTCAGGCTATC	

Genome-wide expression analyses and bioinformatics

Affymetrix gene array analysis of acute liver damage (CCl_4 and partial hepatectomy (pHx)) was performed using the Affymetrix GeneChip® Mouse Genome A430 2.0 (Santa Clara, CA, USA). Detailed analysis was described in,[2]. In addition, mRNA from NASH livers (STAM mice) was retrieved and Affymetrix gene expression analysis was done using Affymetrix GeneChip® Mouse Gene 2.0 ST Arrays (902118).

Immunohistochemistry and immunofluorescent staining

Liver sections of 4 µm thickness fixed in 4% paraformaldehyde buffered with PBS were used for staining. Heat-induced antigen retrieval was either performed in EDTA or Tris-NaCitrate-Dihydrate buffer and endogenous peroxidase activity was blocked using Dako blocking reagent. Primary antibody (αSMA ab5694, Abcam; Digoxigenin ab76907, Abcam; TGF-β2 ab36495, Abcam; CD45 ab10558, Abcam; CK19 #149657-AP, Proteintech ; Pan-cytokeratin (panCK) Z0622, Dako; and Sox9 AB5535, Merck Milipore) incubation was performed at 4°C overnight followed by streptavidin-conjugated horseradish peroxidase antibody incubation. Staining was visualized with diaminobenzidine (DAB) and counterstained with hematoxylin. Quantification (determination of the percentage of stained area) was performed from 5-9 medium-sized non-overlapping 10x magnified fields of each liver sample and expressed as mean percentage ± SEM of the total liver area using ImageJ software 1.48v.

Biodistribution of the AON in the liver was analyzed performing immunofluorescent co-localisation of digoxigenin- (in MDR2-KO) or FAM-labeled (in Balb/c) AONs and specific liver cell type markers, i.e. desmin (ab15200, Abcam), αSMA (ab5694, Abcam), S100A4 (FSP1) (A5114, Dako), elastin (orb13391, Biorbyt) CD31 (ab28364, Abcam) CK19 (Proteintech 149657-AP) and EpCam (Abcam ab71916) of cryo sections. Nuclei were stained with TO-PRO®-3 (T3605, Thermo Fisher Scientific). Secondary antibodies for immunofluorescence analyses were Cy™3 AffiniPure F(ab')$_2$ fragment donkey anti-rabbit IgG (#711-166-152, Jackson Immuno Research Laboratories), Cy™3 AffiniPure F(ab')$_2$ fragment donkey anti-mouse IgG (#715-166-151, Jackson Immuno Research Laboratories), Alexa Fluor® 488 AffiniPure F(ab')$_2$ fragment donkey anti-rabbit IgG (#711-546-152, Jackson Immuno Research Laboratories) and goat anti-rabbit IgG highly cross-adsorbed secondary antibody, Alexa Fluor Plus 555 (Cat #: A32732, Invitrogen).

pSR, αSMA, CD45, F4/80 and CK19 quantification was performed by determination of the percentage of stained area in 6 medium-sized non-overlapping 10x magnified fields of each liver sample and expressed as mean percentage ± SEM of the total liver area using ImageJ software 1.48v. In order to quantify Ki-67 positive hepatocytes (HC) and non-parenchymal cells (NPCs), the number of positive nuclei was counted in 6 representative images per liver/mouse. Average number of positive cells per liver/mouse liver is

presented. Analysis was performed using 4-6 mice per group. To quantify positive PanCK, EpCam and Sox9 signals, 5 portal fields per mouse were randomly chosen. Then number of positive bile ducts (BD) were counted. Analysis was performed using 4-6 mice per group.

Immunoblot

Approximately 10-20 mg of liver tissue were transferred to 2 ml Precellys® tubes filled with 1.4 mm and 2.8 mm ceramic beads and ice-cold RIPA buffer (supplemented with PIC and PMSF). Samples were then homogenized using Precellys® Evolution device at 5000 rpm for 40 s (one cycle). After 30 min incubation on ice lysates were centrifuged and supernatants used for BC Protein assay (Bio-Rad) protein measurement in a microplate reader (Tecan). 20 µg protein lysate were separated using the NuPAGE® Novex® 4-12% Bis-Tris Gel and system and immobilized on nitrocellulose membranes. Membranes were blocked with 5% non-fat dried milk and incubated with aSMA antibody (ab5694, Abcam), PCNA (ab#2586S, Cell signaling), GAPDH (ab9485, abcam) as a loading control overnight at 4ºC. HRP-conjugated secondary antibodies were anti-rabbit IgG-HRP secondary antibody (sc-2301, Santa Cruz Biotechnologgy). Protein detection was performed using Western Lightning® Plus-ECL Substrate.

Picro-Sirius Red Staining for Collagen

For the detection of collagen, paraffin-embedded liver sections were stained with Sirius red as described in,[7 8].

Hydroxyproline assay

Liver collagen content was quantified colorimetrically as total hydroxyproline (HYP) as described elsewhere,[9 10].

In situ hybridization

In situ hybridization of TgfB2-mRNA was performed as described,[11] using the following nucleotide sequence as probe:

Mouse:

forward: CAGTGAATTGATTTAGGTGACACTATAGAAGTGGCTTCACCACAAAGACAGGAACCTG;

reverse: CAGTGAATTGTAATACGACTCACTATAGGGAGAAGGTGAGATGCAGACTAACGCCTTC.

TgfB2 mouse NCBI Reference Sequence is NM_001329107.1

Human:

forward: TGCTCTGTGGGTACCTTGATGCCAT

reverse: CGGTTGGTCTGTTGTGACTCAAGTC

TGFB2 human NCBI Reference Sequence is NM_001135599.2

High-throughput gene expression analysis

High-throughput gene expression analysis was performed by real-time PCR on Fluidigm's Biomark HD quantitative chip platform (Fluidigm) as described in Dropmann et al.,[12].

FACS analysis

Livers of 6 control oligo and 6 AON treated MDR2-KO mice (treatment as described in Materials and Methods section AON treatment of MDR2-KO mice (cholestatic fibrosis model) were perfused with PBS, dissected and processed in digestion buffer using gentleMACS™ Dissociator (Miltenyi Biotec). Immune cells were enriched by centrifugation in 33% Percoll (GE Healthcare), counted and used for analysis by flow cytometry. Briefly, dead cells were labelled with Zombie Aqua™ fluorescent dye (Biolegend), followed by incubation with a supernatant of a CD16/32-producing hybridoma cells. Upon staining of surface markers, cells were fixed and permeabilized using eBioscience™ Foxp3 / Transcription Factor Staining Buffer Set (Thermo Fisher), and

intracellular staining was performed according to manufacturer's instructions. Antibodies for surface and intracellular staining were purchased form BD and Biolegend.

SUPPLEMENTAL FIGURE LEGENDS

Supplemental Figure 1: *TfgB2* expression in non-biliary derived mouse liver damage. *TgfB2* expression was not changed significantly (A) in C57BL6/j STAM mice, (B) after partial hepatectomy (PHx) or (C) upon acute or (D) chronic CCl_4 treatment of C57BL/6N mice. h=hours, d= days, w = weeks, m=months

Supplemental Figure 2: *TGFB2* expression in patients with liver disease of different etiology. *TGFB2* expression was not significantly altered in steatosis, NASH and NAFLD patient cohorts (A) E-GEOD-59045, (B) E-GEOD-48452, (C) E-GEOD-61256, (D) E-MTAB-4856. (E) In GSE31803 advanced fibrosis samples, higher *TGFB2* levels were detected as compared to mild fibrosis. (F) However, significant elevation of *TGFB2* was detected in alcoholic hepatitis (AH) as compared to control tissue in the cohort E-GEOD-28619. * $p \leq 0.05$, **** $p \leq 0.0001$.

Supplemental Figure 3: Patient resolved expression of *TGFB2* in tissue of PSC and PBC patients compared to healthy individuals separated by entity.
Expression changes of *TGFB2* in liver tissue of individual patients represented as single dots (A) in the PSC/PBC cohort from Regensburg (B) in the collective GSE61260. PSC and PBC patients are presented separately.

Supplemental Figure 4: **Design of *TgfB2*-directed AONs.** (A) *TgfB2*/*TGFB2* complementary sequence of the *TgfB2*-directed AON ISTH0047 which was finally engineered as a 17-mer full phosphorothioate LNA-modified antisense oligodeoxynucleotide '4+4' gapmer. A scramble control oligo C3_0047 (Co) was used to control for nonsequence-specific biological effects of oligonucleotides.

Supplemental Figure 5: (A) Liver parameters of Balb/c and MDR2-KO mice after administration of control oligos (Co) as compared to *TgfB2*-specific AONs (AON) compared to untreated (ut) animals. 15mg AON/kg body weight were administered. ALT, AST and Alkaline phosphatase levels were higher in MDR2-KO mice than in Balb/c wild-type animals. Treatment with specific or control AON did not alter liver parameters. (B)

The body weight of all animals was not altered by treatment with control or *TgfB2*-specific AONs. ** $p \leq 0.01$, *** $p \leq 0.001$, ****$p \leq 0.0001$.

Supplemental Figure 6: Localization of *TgfB2*-directed AONs by immunohistochemistry and co-immunofluorescence and *TgfB2* expression in liver cell types. In wild-type and MDR2-KO mice, the *TgfB2*-specific AONs were enriched in sinusoidal areas of the liver tissue as confirmed by (A) immunohistochemical staining of Digoxigenin-labelled AONs (first panel). (B) Immunofluorescence revealed colocalization of the AONs (red) with non-parenchymal cell markers, i.e. αSMA, desmin, elastin, S100A4 and CD31 (each green). No co-localization was detected with the cholangiocyte marker CK19. Hepatocyte nuclei are stained in blue by TO-PRO®-3. White scale bars indicate 50µm, red scale bars indicate 25µm. The second and forth column represent magnifications of parts of the image in column 1 and 3, respectively, to highlight representative stainings. (C) *TgfB2* was expressed in non-parenchymal liver cells, i.e. HSCs, LSECs, and KC as confirmed by qPCR following 4 cell type-specific cell isolation from Balb/c and MDR2-KO mouse livers. Relative expression was referred to the mean value of all cell types in Balb/c. Error bars indicate SD.

Supplemental Figure 7: Regulation of *PparG* in JS-1 cells upon TGF-β2 treatment. According to a pro-fibrotic role of TGF-β2, TGF-β2 treatment of JS-1 cells inhibited *PparG* expression. Error bars indicate SD.

Supplemental Figure 8: FACS analysis of the myeloid compartment revealed increased F4/80 expression and eosinophil frequencies in AON-treated MDR2-KO mice (A) Histograms depict F4/80 expression on gated myeloid cells (CD3/CD19/NKp46/DX5negCD11b+) in livers of 3 representative control oligo or AON treated MDR2-KO mice. Graphs show quantification of F4/80 expression and frequency of F4/80-expressing cells within gated myeloid compartment of 6 control oligo treated and 5 AON treated MDR2-KO mice, **p<0.01, Mann-Whitney test. (B) Dot-plots displaying gated myeloid cells in livers of representative control oligo or AON treated MDR2-KO mice, depicting Ly6G+SiglecF neg neutrophils (left) and SiglecF and F4/80 expressing eosinophils (middle). The remaining cells comprise mainly Ly6C expressing

monocytes (right). (C) Gating strategy used for analysis of myeloid and lymphoid cells in liver. (D) Fold change of absolute cell numbers per mg of liver tissue of indicated immune cell subsets relative to the mean value of control-treated animals (left), and absolute CD8+ T cell numbers per mg of liver tissue, as well as their frequencies among total immune cells (right) are shown. *p<0.05, Mann-Whitney test.

SUPPLEMENTAL TABLES

Supplemental Table 1: Correlation between *TGFB2* expression and clinicopathological characteristics of 15 PSC/PBC patients analyzed from the Regensburg collective. Expression levels of *TGFB2* mRNA were compared to 5 normal human liver samples. By Fisher's exact test a significant correlation with inflammation was observed ($p \leq 0.05$). No other correlation with age, gender, grade of steatosis and fibrosis was observed. High *TGFB2* was defined as a fold change of > 1.5, low was defined as less.

Correlation of high vs low *TGFB2* expression with clinical characteristics			
Clinicopathological parameters	**High *TGFB2* (n=11,%)**	**Low *TGFB2* (n=4,%)**	***p*-value**
Age (years)			**1**
<50	4 (36.36)	1 (25)	
≥50	7 (63.64)	3 (75)	
Gender			**1**
Male	4 (36.36)	1 (25)	
Female	7 (63.64)	3 (75)	
Type of fatty degeneration			**1**
0	8 (72.73)	3 (75)	
3	1 (9.09)	0 (0)	
4	2 (18.18)	1 (25)	
Grade of steatosis			**1**
0	9 (81.82)	3 (75)	
4	2 (18.18)	1 (25)	
Fibrosis			**0.28**
0	6 (54.55)	1 (25)	
1	1 (9.09)	0 (0)	
3	1 (9.09)	1 (25)	
4	3 (27.27)	2 (50)	
Inflammation			**0.045**
0	2 (18.18)	3 (75)	
1	2 (18.18)	1 (25)	
4	7 (63.64)	0 (0)	

Supplemental Table 2: ***TGFB2*** **expression changes in the PBC cohort GSE79850 comparing high and low risk patients to non-diseased control livers.**

Samples		Etiology	GEO2R Analysis	adj. *p*-value	Mean log2 (Fold Change)
9 High risk and 7 Low risk patients	8 control livers	high risk (n=9, who went on to require liver transplantation) and low risk (n=7, who responded fully to UDCA) patient material was processed along with non-diseased control liver (n=8)	**High vs Control**	**0.004556**	2,089
			Low vs Control	**0.217**	-0.55
			High vs low	**0.0026**	2.645

Number and classification of patients, *p*-values and fold change of *TGFB2* expression in the patient cohorts are listed. High *TGFB2* is associated with bad prognosis (liver transplantation) instead of therapy response.

Supplemental Table 3: Clinicopathological characteristics of 17 PBC patients included in GSE79850.

clinicopathologic characteristics	(n= 17)
Scheuer grade	
I+II	7
III+IV	7
Portal inflammation	
none	3
mild	7
moderate	7
Interface hepatitis	
none	9
mild	4
moderate	4
Ductopenia	
(+)	9
(–)	8

Supplemental Table 4: **Correlation between *TGFB2* expression and clinicopathological characteristics of the 17 PBC patients analyzed and described in GSE79850.** Expression levels of *TGFB2* mRNA were compared between high risk patients, who underwent liver transplantation as in comparison to low risk patients displaying full response to UDCA treatment. By Fisher's exact t-test, significant correlations were observed with Scheuer grade and Ductopenia ($p \leq 0.05$). No correlation with portal inflammation and diagnosis of interphase hepatitis was observed.

Correlation of high risk vs low risk classification with clinical characteristics			
	High risk (n=)	**Low risk (n=)**	***p*-value**
Scheuer grade			**0,0047**
I+II	1	6	
III+IV	7	0	
Portal inflammation			0,1026
mild	2	5	
moderate	6	1	
Interface hepatitis			0,5804
none-mild	5	5	
moderate	3	1	
Ductopenia			**0,0256**
(+)	7	1	
(–)	1	5	

Supplemental Table 5: Inflammation- and fibrosis-associated marker genes regulated by AON-treatment in MDR2-KO mice. Selection criteria were applied as follows: 1. Log2FC in AON-treated MDR2-KO mice (AON) compared to untreated MDR2-KO mice (ut) was -0.5<Log2FC>0.5 and 2. *p*-values ≤ 0.05, i.e. * ≤0.05, ** ≤0.01, *** ≤0.001, ****<0.0001 comparing AON with ut. *p*-values comparing AON with control oligo treated MDR2-KO mice (Co) and comparing Co with ut are also given for these genes. Ct-values were normalized to beta actin and untreated animals of MDR2-KO, using the ΔΔCt-method ,[6]. Green: *p*-values ≤ 0.05 for ut vs AON and ut vs Co, i.e. Placebo effect; Red: *p* ≤ 0.05 for ut vs AON and Co vs AON, i.e. significant AON effect. ns: not significant.

		ANOVA *p*-values		
targets	**Log2FC**	**ut vs AON**	**Co vs AON**	**ut vs Co**
Ifnb1	-7,0071	****	ns	****
Ch25h	-6,9784	****	ns	****
Ifna1	-6,7997	****	ns	****
Acta2	-6,4616	****	ns	****
Cxcr1	-6,2092	****	ns	****
Ccr5	-5,8665	****	ns	****
Ccr3	-4,6940	****	ns	****
Ccr2	-2,6257	****	ns	****
TgfB2	-1,3555	ns	**	ns
Gapdh	-1,1487	****	ns	***
Cav1	-0,9961	ns	ns	ns
Egfr	-0,9728	ns	ns	ns
Rps18	-0,9208	***	ns	*
Col4a3	-0,6004	ns	ns	ns
Twist1	-0,5664	ns	ns	ns
CD133	0,5132	ns	ns	ns
Egf	0,5154	ns	ns	ns
Nes	0,5392	ns	ns	ns
TgfB1	0,5903	**	ns	ns
Smad7	0,5933	ns	ns	ns
Col1a1	0,6561	ns	ns	ns
Notch1	0,6594	*	ns	**
TbetaRII	0,7012	ns	ns	***
Il10rb	0,7361	***	ns	**
BMP-9	0,7535	*	ns	ns
SparcA	0,7715	*	ns	ns
Mrc1	0,8170	*	ns	*
Mki67	0,8279	*	*	ns
Cxcl10	0,8891	ns	ns	ns

Birc5	0,9568	*	ns	ns
Notch3	0,9896	***	**	ns
Cyp2e1	1,0137	**	ns	*
Ifng	1,2211	ns	ns	ns
Lama1	1,3519	****	*	*
Ccl3	1,4114	****	**	ns
Ccl4	1,5082	****	***	ns
Ccl5	1,5261	***	***	ns

Supplemental Table 6:

Inflammation- and fibrosis-associated marker genes were chosen as described by Abshagen et al. ,[13].

Acta2	*Cdh2*	*Ifna1*	*Pde4a*
Alb	*Ch25h*	*Ifnb1*	*Pde4b*
Bad	*Col1a1*	*Ifng **	*Pde4d*
Bax	*Col3a1*	*Igf1*	*Pdgfb*
Bcl2/Bak1	*Col4a3*	*Il10rb*	*Pparg*
beta-actin	*Col6a6*	*Il12a **	*Prrx1*
Bim/Bcl2l11	*Col8a1*	*Il13 **	*Pten*
Birc5	*Ctgf*	*Il1b*	*Rarres1*
BMP-9/Gdf2	*Cxcl1*	*Il1rn*	*Rps18*
Cat	*Cxcl10*	*Il2 **	*Six1 **
Cav1	*Cxcl12*	*Il4 **	*Smad6*
Ccl3	*Cxcl2*	*Il6 **	*Smad7*
Ccl4	*Cxcl3 **	*Il6st*	*SparcÂ*
Ccl5	*Cxcl5*	*Lama1*	*TbetaRII/Tgfbr2*
Ccl7	*Cxcr1*	*Met*	*Tgfb1*
*Ccl8 **	*Cxcr2*	*Mki67*	*Tgfb2*
Ccr2	*Cyp2e1*	*Mmp10 **	*Timp1*
Ccr3	*Edn1*	*Mmp9*	*Timp2*
Ccr5	*Egf*	*Mrc1*	*Tnc*
CD133/Prom1	*Egfr*	*Nes*	*Tnf*
Cd14	*FasL*	*Notch1*	*Tnfrsf1a*
Cd69	*Fn1*	*Notch3*	*Twist1*
Cd86	*Gapdh*	*Osm **	*Wisp1*
Cdh1	*Hgf*	*Osmr*	*Xiap*

*: no Ct values obtained and thus excluded from the evaluation

SUPPLEMENTAL REFERENCES

1 Guo J, Loke J, Zheng F, et al. Functional linkage of cirrhosis-predictive single nucleotide polymorphisms of Toll-like receptor 4 to hepatic stellate cell responses. *Hepatology* 2009;49(3):960-8.
2 Godoy P, Widera A, Schmidt-Heck W, et al. Gene network activity in cultivated primary hepatocytes is highly similar to diseased mammalian liver tissue. *Arch Toxicol* 2016;90(10):2513-29.
3 Hammad S, Braeuning A, Meyer C, et al. A frequent misinterpretation in current research on liver fibrosis: the vessel in the center of CCl4-induced pseudolobules is a portal vein. *Arch Toxicol* 2017;91(11):3689-92.
4 Saito T, Muramatsu M, Ishii Y, et al. Pathophysiological analysis of the progression of hepatic lesions in STAM mice. *Physiol Res* 2017;66(5):791-99.
5 Wasik U, Milkiewicz M, Kempinska-Podhorodecka A, et al. Protection against oxidative stress mediated by the Nrf2/Keap1 axis is impaired in Primary Biliary Cholangitis. *Sci Rep* 2017;7:44769.
6 Livak KJ, Schmittgen TD. Analysis of relative gene expression data using real-time quantitative PCR and the 2(-Delta Delta C(T)) Method. *Methods* 2001;25(4):402-8.
7 Puchtler H, Waldrop FS, Valentine LS. Polarization microscopic studies of connective tissue stained with picro-sirius red FBA. *Beitrage zur Pathologie* 1973;150(2):174-87.
8 Junqueira LC, Bignolas G, Brentani RR. Picrosirius staining plus polarization microscopy, a specific method for collagen detection in tissue sections. *The Histochemical journal* 1979;11(4):447-55.
9 Jamall IS, Finelli VN, Que Hee SS. A simple method to determine nanogram levels of 4-hydroxyproline in biological tissues. *Analytical biochemistry* 1981;112(1):70-5.
10 Boigk G, Stroedter L, Herbst H, et al. Silymarin retards collagen accumulation in early and advanced biliary fibrosis secondary to complete bile duct obliteration in rats. *Hepatology* 1997;26(3):643-9.
11 Ghafoory S, Breitkopf-Heinlein K, Li Q, et al. A fast and efficient polymerase chain reaction-based method for the preparation of in situ hybridization probes. *Histopathology* 2012;61(2):306-13.
12 Dropmann A, Dediulia T, Breitkopf-Heinlein K, et al. TGF-beta1 and TGF-beta2 abundance in liver diseases of mice and men. *Oncotarget* 2016;7(15):19499-518.
13 Abshagen K, Konig M, Hoppe A, et al. Pathobiochemical signatures of cholestatic liver disease in bile duct ligated mice. *BMC Syst Biol* 2015;9:83.