Thieme

36. Jahrestagung der Deutschen Arbeitsgemeinschaft zum Studium der Leber

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Kongresspräsident:
Prof. Dr. Peter Galle

e1 Lectures and Posters

Lectures Session I Basic Hepatology (Fibrogenesis, NPC, Transport) Friday, February 14, 2020, 1:25 pm – 2:10 pm, Lecture Hall P1

1.1 The G protein coupled bile acid receptor TGR5 (Gpbar1) modulates endothelin-1 signaling in liver

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Introduction TGR5 (Gpbar-1) is a G-Protein-coupled bile acid (BA) receptor (Kawamata et al. 2003) expressed on nonparenchymal liver cells such as hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs) (Keitel et al. 2006, 2008). Lithocholic acid (LCA) is a hydrophobic bile acid. Feeding mice with a diet containing 1%LCA leads to the development of a severe toxic liver injury (Fickert et al. 2006). The most relevant complication of liver damage is the development of portal hypertension (PH). It has been demonstrated previously that activated HSCs contribute to PH by Endothelin-1 (ET-1)-induced cell-contraction in a cAMP-dependent manner (Rockey et al 1993, Reinehr et al. 2002). If TGR5 plays a role during this process was unknown.

Methods 8 – 12 week old wildtype (WT) and TGR5 knockout (KO) mice were sacrificed after receiving a diet containing 1%LCA for 84 hrs. Liver damage was determined via analysis of liver enzymes in serum and HE-staining. Portal pressure was measured by cannulation of the portal vein. Hepatic mRNA-expression of different genes was determined by RT-PCR analysis. HSC contraction was tested using an HSC-contraction assay. Localization of the ETA-receptor on HSCs was analyzed by immunofluorescence (IF) and colocalization-analysis. ET-1 levels in LSECs were measured via ELISA-immunoassay and RT-PCR.

Results TGR5 KO mice suffered from a more severe liver damage as compared to WT animals when fed a 1%LCA diet as determined by the area of necrosis seen in liver tissue as well as increased serum AST-levels. Measurement of the portal pressure in these mice revealed an induction of PH in LCA-fed KO mice but in none of the control groups. RT-PCR-analysis confirmed an increased expression of genes associated with PH, e.g. ET-1 in livers of LCA-fed TGR5 KO mice. Challenge of LSECs as the most important source of ET-1 in the liver with a TGR5 agonist caused a significant reduction of ET-1 secretion. Moreover, treatment of mice and isolated HSCs with a TGR5 agonist was able to attenuate ET-1-induced PH as well as HSC contraction, respectively. By

means of IF of the ETA-receptor on the surface of HSCs and colocalization analysis we were able to demonstrate internalization of this receptor after treatment with a TGR5 agonist.

Conclusion In conclusion, TGR5 plays a protective role against the development of PH after induction of liver injury by two synergistically acting mechanisms.

1.2 Intestinal dysbiosis amplifies acetaminophen induced acute liver injury

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Question Acute liver failure (ALF) represents an unmet medical need in western countries. Here Acetaminophen (APAP) poisoning and infections are major causative agents. While the link between intestinal dysbiosis and chronic liver disease is well shown by numerous studies, there is little evidence for a functional link of gut-liver interaction during ALF. Here, we hypothesized that intestinal dysbiosis may affect the outcome of ALF.

Methods Male 6 – 8 week old wildtype (WT) and dysbiotic Nlrp6$^{-/-}$ mice were injected with a sublethal dose of APAP or lipopolysaccharide (LPS) to induce ALF. APAP metabolism was analyzed at different time points after injection. 12 hours after injection, liver injury was comprehensively analyzed based on liver functions tests (LFTs), histology, flow cytometry immunophenotyping (FACS) and 16S rRNA-based microbiota profiling. Furthermore changes in the gut barrier function were studied and microbiota of WT, Nlrp6$^{-/-}$, TLR4$^{-/-}$ and TLR9$^{-/-}$ mice was modulated by fecal microbiota transfer (FMT) before ALF induction.

Results Dysbiotic Nlrp6$^{-/-}$ mice showed significantly increased liver injury upon APAP and LPS treatment compared to WT controls, which was evidenced by LFTs, hepatic necrosis quantification and inflammation. While we did not observe major changes in APAP metabolism, enhanced liver damage in Nlrp6$^{-/-}$ mice was associated with markedly increased infiltration of Ly6C^{hi} monocyte derived macrophages (MoMFs) as demonstrated by FACS analysis. In WT mice, ALF induced a shift in microbiota composition and an expansion of colonic mucus layers. This protective response was absent in Nlrp6$^{-/-}$ mice, prompting increased serum endotoxin levels after APAP administration. Remarkably, fecal microbiota transfer (FMT) from Nlrp6$^{-/-}$ mice into WT mice aggravated liver injury upon APAP treatment in WT mice, resembling the inflammatory phenotype of Nlrp6$^{-/-}$ mice. Specifically, after FMT of dysbiotic microbiota monocyte polarization in WT mice was skewed toward a Ly6C^{hi} inflammatory phenotype suggesting a critical role of these cells within gut-liver axis as sensors of gut-derived signals shifting the inflammatory response in the liver.

Conclusions Our data show a crucial, so far unknown function of intestinal microbiota during ALF. Intestinal dysbiosis of Nlrp6$^{-/-}$ mice was transferrable to healthy WT controls via FMT, promoted pro-inflammatory Ly6C^{hi} macrophage polarization and augmented liver injury.

1.3 YAP-induced tumor initiation mediates a switch in hepatic macrophage identity via the Ccl2/Ccr2 axis

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Background The development of hepatocellular carcinoma (HCC) precursor lesions includes the functional modulation of disease modifying non-parenchymal cells (NPCs). However, how oncogenes affect heterologous communication between tumor cells and NPCs is not well defined. Here, the impact of *yes-associated protein* (YAP)-induced chemokines in tumor initiation on liver macrophages is analysed.

Methods Transgenic mice with liver-specific expression of constitutively active YAP (LAP-tTA/YAP$^{S\,127A}$) were used as HCC model. Immunofluorescence for Kupffer cell (KC) markers (Clec4f, F4/80) and a bone-marrow-derived macrophage (BMDM) marker (CD11b) were performed on mouse tissue specimens and correlated with vascular morphometric data. Murine blood plasma, primary hepatocytes and CD11b^{+} F4/80^{+} retinoid^{-} macrophages from healthy and HCC precursor-bearing livers were used for proteomic and transcriptomic profiling. Immortalized THP-1 and SK-Hep1 cell lines served as in vitro model systems (e.g. chromatin-immunoprecipitation; ChIP). Human HCC expression data and tissue microarrays were analysed.

Results Bioinformatic analysis revealed that YAP$^{S\,127A}$ expressing hepatocytes showed a significant induction of a chemokine gene cluster. Increased *CC-chemokine ligand 2* (Ccl2), *CC-chemokine ligand 5* (Ccl5) and *Macrophage colony-stimulating factor 1* (Csf1) levels were confirmed on protein levels in blood plasma. Ccl2 increased transmigration of macrophages and was transcriptionally regulated by the YAP/TEAD4 complex. Livers from YAP$^{S\,127A}$ mice were characterized by an increased macrophage recruitment, which lacked the KC phenotype. Importantly, these macrophages were defined by a lack of functional polarization (M0 signature), extravascular localization and high *C-C motif chemokine receptor 2* (Ccr2) expression. Vascular morphometry revealed an increased branch length, junction formation and elevated vessel diameters in YAP$^{S\,127A}$ transgenic mice that correlated with the degree of macrophage infiltration. Lastly, the presence of an M0 signature was also detectable in human HCC and served as an identifier for poor clinical outcome.

Conclusion YAP-induced tumor initiation results in increased BMDM recruitment leading to a population-based phenotypic switch of the liver macrophage compartment. The Ccl2/Ccr2 axis directly governs the perivascular infiltration of macrophages to preneoplastic lesions where these cells contribute to vascular remodelling.

Lectures Session II Clinical Hepatology, Surgery, LTX
Friday, February 14, 2020, 3:25 pm – 4:10 pm, Lecture Hall P1

2.1 Safety and Efficacy of 10 mg Myrcludex B/IFNa Combination Therapy in Patients with Chronic HBV/HDV Co-Infection

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Background Myrcludex B/Bulevirtide (BLV) is a first-in-class entry inhibitor to treat HBV/HDV co-infected patients. BLV monotherapy, as well as combination with Tenofovir (TDF) or PEG-interferon α-2a (PEG-IFNα) induced serum and intrahepatic HDV RNA decline in two phase 2 clinical trials (MYR202/203). 2 mg and 5 mg BLV as combination therapy with PEG-IFNα showed strong synergistic effects on HDV RNA and induced HBsAg reduction. In a substantial number of patients HBsAg was undetectable and seroconversion occurred. We here present results of an extended 48 week phase 2 study (MYR203 extension) on HBV/HDV patients receiving 10 mg BLV in combination with PEG-IFNα or TDF.

Methods 30 HBeAg-negative patients with chronic HBV/HDV co-infection were randomized in 2 arms. Over 48 weeks 10 mg BLV was subcutaneously injected once daily in combination with 180 µg PEG-IFNα once weekly or 5 mg BLV was administered twice daily (total daily dose of 10 mg BLV) with TDF. At baseline all patients were positive for HDV serum RNA with median viral loads of 6.28 log10 IU/ml in the 10 mg BLV/PEG-IFNα group and 6.24 log10 IU/ml in 10 mg BLV/TDF group. Robogene assay was employed to determine HDV RNA (LOD: 10 IU/ml).

Results Safety: Over 24 weeks BLV was well tolerated and no serious adverse event (SAE) was reported. Altogether 358 AEs were reported: two thirds were considered to be related to PEG-IFNα (n = 191) or TDF (n = 24). One third was considered to be related to BLV (mild n = 71, moderate n = 39, severe n = 11) and caused by total bile salt increase. Efficacy: Serum HDV RNA levels declined with median reductions from baseline by -4.84 log10 IU/ml in the BLV+PEG-IFNα arm (n = 15) and -2.80 log10 IU/ml in the BLV+TDF arm (n = 14) after 24 weeks. HDV RNA was undetectable in 60% and 21% patients, respectively. 20% of patients achieved ALT normalization by combination with IFN compared to 57% with TDF. HBsAg declined by > 1log10 IU/ml in two patients but was detectable further on.

Conclusion Over a period of 24 weeks 10 mg BLV in combination with PEG-IFNα or TDF is safe and well tolerated. The combined treatment with IFNa exhibits a strong synergistic effect on the antiviral response against HDV. Besides, administration of 5 mg BLV twice daily is not superior to 10 mg BLV once daily. End-of-treatment data (48 weeks) will be presented at the meeting.

2.2 Activin-driven fate determination is pivotal for liver progenitor cells to take over hepatocytic function in in ACLF

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DOI 10.1055/s-0039-3402106

Background & Aim Liver progenitor cell (LPC) differentiation into mature hepatocytes determines survival and recovery of patients with acute-on-chronic liver failure (ACLF), who suffer from massive hepatic necrosis. Interestingly, even in irreversible ACLF, LPCs differentiate towards hepatocytes. However, these LPC-derived hepatocytes do not provide sufficient hepatocytic function, as required for systemic homeostasis. This study demonstrates a crucial role of Activin-dependent lineage-determining transcription factor HNF4a in the maintenance of key hepatocyte function.

Methods This study enrolled 28 ACLF patients (20 receiving liver transplantation and 8 spontaneously recovered). Expression of transcription factors in liver tissues was measured by immunohistochemistry. Murine LPCs were used to investigate the regulatory mechanisms underlying HNF4a expression.

Results: *In vitro*, LPCs express HNF4a and key hepatic functional genes/proteins, e.g. albumin and coagulation factor V (f5). Knockdown of HNF4a remarkably reduces expression of these genes/proteins. ChIP assays confirm that HNF4a controls albumin and f5 gene expression by binding to their promoters. Activin, but not TGF-β, induces HNF4a expression in LPCs. Co-IP analyses further indicate that Activin-mediated HNF4a expression requires formation of two transcription factor complexes, TRIM33-SMAD2/3 and FOXH1-SMAD2/3/4. Knocking down any components interferes with HNF4a and target gene expression. Interestingly, Activin administration or depletion of HNF4a does not alter the epigenetic phenotype of LPCs, e.g. H3K4me3, H3K27me3 and H3K27ac expression, indicating that the Activin-HNF4a axis does not induce cell reprogramming. Consistent with the *in vitro* findings, 8 recovered ACLF patients display robust expression of p-Smad2, TRIM33, FoxH1 and HNF4a in LPC nuclei, whereas irreversible ACLF patients lost multiple or all transcription factors. We found a negative association between HNF4a expression and MELD scores, that is, all recovered patients displayed both high levels of HNF4a and low MELD scores. In contrast, none of the irreversible ACLF patients demonstrated robust HNF4a expression in LPCs. In ACLF patients, expression of H3K4me3, H3K27me3 and H3K27ac was not relevant toHNF4a expression.

Conclusions These results highlight a key mechanism of Activin signal-driven lineage determination for avoiding liver failure. Targeting the Activin-HNF4a axis might provide a novel approach in ACLF treatment.

2.3 SEAL program – Early detection of liver fibrosis and cirrhosis by screening of the general population

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DOI 10.1055/s-0039-3402107

Background Most patients with liver cirrhosis are detected at late stages of their disease. In approximately 75% of cases diagnosis is based on the development of complications such as ascites or bleeding. At this stage causative treatment is less successful or impossible. Screening for liver health and disease is not included in standard medical check-up programs. The SEAL program (Structured Early Detection of Asymptomatic Liver Cirrhosis) investigates the feasibility, effectiveness and cost-benefit assessment of a general screening program for liver fibrosis and cirrhosis.

Method As part of the G-BA-funded SEAL program, 16,000 insured persons from a large German health insurance company (AOK) are offered additional testing of liver enzymes as part of the nationwide primary care program (check-up 35) in two German states, i.e. Rhineland-Palatinate and Saarland. In case of elevated serum aminotransferase activities, the APRI score as a liver fibrosis risk marker is calculated. If the APRI score is suggestive for liver disease, patients are referred to a gastroenterologist for further differential diagnostic assessment. Patients suspected to have relevant liver fibrosis present at

an academic medical center for further workup. Endpoints include data on the epidemiology of elevated liver enzymes, cost-benefit assessments and prevalence rates of liver fibrosis in the general population.

Results To date, more than 7.000 patients have been enrolled in the SEAL program. The average age of female and male patients was 64 years and 62 years, respectively. Of the examined patients, 11% and 6% presented with increased ALT and AST activities with a maximum of 1.050 U/l and 434 U/l, respectively. In 499 patients, known liver disease was present at inclusion. Nevertheless, 11% and 5% of patients without known liver disease presented with significantly elevated ALT and/or AST activities, triggering further dedicated examinations by liver experts.

Conclusions The establishment of an early diagnosis of liver diseases is controversially discussed by professionals and in the literature. SEAL data already indicate that a relevant group of the population presents with increased aminotransferases but no diagnosis of chronic liver disease. The SEAL project will provide data on feasibility, effectiveness and cost-benefit assessment in our health care system and provide evidence for the implementation of such a screening measure.

Lectures Session III Metabolism (incl. NAFLD) Friday, February 14, 2020, 6:20 pm – 7:05 pm, Lecture Hall P1

3.1 Maternal exercise conveys protection against NAFLD in the offspring via hepatic metabolic programming

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DOI 10.1055/s-0039-3402108

Background and aims Maternal exercise (ME) during pregnancy has been shown to improve long-term metabolic health in offspring. However, the effects of ME on the development of non-alcoholic fatty liver disease (NAFLD) in offspring and its underlying mechanisms remain poorly understood.

This study aimed at determining the long-term effects of ME during pregnancy on offspring body composition and development of NAFLD while focusing on proteomic-based analysis of the hepatic energy metabolism during postnatal developmental organ programming.

Methods C57BL/6N Mouse dams were divided into a sedentary control group (CO) and a running intervention group (INT), which performed voluntary wheel running throughout gestation. At postnatal day (P)70, half of the offspring of both groups was challenged with a high fat diet (HFD) for the following six weeks until P112 (CO-HFD and INT-HFD). Male offspring was sacrificed at weaning (P21) and in later life (P112) and profiling of hepatic key metabolic processes was conducted. At P21, liver proteomic screens and at P112 liver histomorphology were performed.

Results Offspring of exercised dams were protected from HFD-induced body weight gain and NAFLD in later life (P112). This was associated with a significant activation of hepatic AMP-activated protein kinase (AMPK), peroxisome proliferator-*activated* receptor alpha (PPARα) and PPAR coactivator-1 alpha (PGC 1α) signaling and with reduced hepatic lipogenesis and increased intrahepatocellular β-oxidation at a critical point in time of developmental programming in early life (P21). Concomitant proteomic analysis revealed a characteristic hepatic expression pattern in the offspring as a result of ME with the most prominent impact on Cholesterol 7 alpha-hydroxylase (CYP7A1).

Conclusion:ME may offer protection against offspring HFD-induced NAFLD by shaping hepatic proteomics signature and metabolism in early life. The results highlight the potential of exercise during pregnancy for antagonizing the early origins of NAFLD.

3.2 A novel receptor for bile salts – the G protein-coupled receptor MRGX4 is expressed on sensory neurons

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DOI 10.1055/s-0039-3402109

Background&Aims Mas gene-related G protein-coupled receptors (Mrg) represent a class of pruriceptors expressed on sensory neurons. Pruritus is a frequent symptom in various hepatobiliary disorders. The recent beneficial effects of ileal bile acid transport inhibitors in patients suffering from cholestatic pruritus have raised interest in bile salts as pruritogens. We hypothesized that cholephilic substances in serum of cholestatic patients may activate MRG receptors thereby causing pruritus.

Methods Human and murine MRG receptors were cloned and stably expressed in HEK293 cells. Activation of MRG receptors in HEK293 or DRGs by cholephilic substances were measured by changes in cytosolic free calcium $(Ca^{2+})_i$ using ratiometric fluorimetry. In mice scratch activity after intradermal pruritogen injection was quantified observer-independently measuring electric fields induced by implanted magnets. In 15 healthy volunteers itch intensity was quantified on a numeric rating scale after intradermal injection or focal application.

Results We investigated the potential of human cholestatic serum and bile for activation of human and murine MRG receptors. Fractions containing bile salts resulted in rise of $(Ca^{2+})_i$ in hMRGX4 expressing HEK cells but neither of other human or murine MRG isoforms nor cultures of freshly dissociated murine DRGs. Analyzing human bile salts revealed that certain bile salt species (referred to as hMRGX4-activating bile salt species) were capable of activating hMRGX4 while others did not. The EC50 values ranged within the pathophysiological range of low micromolar concentrations. Intradermally injected bile salts did not cause significant scratching behavior in mice. In contrast, in humans focally applied or intradermally injected hMRGX4-activating bile salts caused significant itch intensity compared to non-hMRGX4 activating bile salts. Laser-doppler imaging indicated no widespread axonreflex erythema excluding relevant mast cells activation.

Conclusions These data unravel a novel signaling pathway for bile salt subspecies that may contribute to cholestatic pruritus and may explain the differences between murine and human cholestasis. The hMRGX4 receptor represents a promising drug target to alleviate cholestatic pruritus.

3.3 Genes involved in hepatic cholesterol homeostasis identified as modifiers of cholestasis in Abcb4 deficient mice

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DOI 10.1055/s-0039-3402110

Background The severity of *ABCB4*-related liver diseases varies with transporter functionality and genetic predisposition. Modifier genes have yet to be investigated. In the *Abcb4*-/- mouse model, deficiency of the hepatobiliary phosphatidylcholine translocase causes chronic cholangiopathy and biliary fibrosis. Recently we identified fibrogenic quantitative trait loci (QTL) in an experimental cross of congenic FVB- and BALB-*Abcb4*-/- knockout strains and potential candidate genes regulating hepatic cholesterol metabolism. Among these we investigated the role of *Pcsk9* (proprotein convertase subtilin-kexin type 9), which controls the degradation of the LDL receptor, in *Abcb4*-/- and wild-type mice. In addition, we analysed the association of *PCSK9* variants in patients with primary sclerosing cholangitis (PSC).

Patients and Methods Hepatic injury was characterized by measurement of collagen accumulation via colorimetric measurement of hydroxyproline and serum surrogate markers (ALT, AP). We determined plasma lipid profiles and hepatic steady-state mRNA expression of *Pcsk9*, *Hmgcr*, *Ldlr* and *Srebp2* as important regulators of cholesterol homeostasis. Genetic variation in the orthologous *PCSK9* locus was tested in a cohort of 193 patients with PSC using Taqman assays.

Results *Pcsk9* expression is significantly ($p = 0.01$) higher in FVB mice as compared to fibrotic BALB-*Abcb4*-/- mice. Irrespective of genetic background, wild-type mice display significantly ($p = 0.01$) higher *Pcsk9* mRNA levels than knockouts. Concomitantly, plasma cholesterol levels are significantly ($p < 0.01$) higher in controls than in *Abcb4* deficient mice and highest in FVB mice. *Hmgcr* expression is significantly ($p < 0.05$) reduced in susceptible BALB-*Abcb4*-/- mice. The genetic analysis of the PSC cohort revealed that carriers of the variant rs562556 *PCSK9* allele presented with significantly ($p = 0.017$) higher serum AP activity, the bona fide surrogate marker of PSC severity.

Conclusions The analysis of ABCB4-dependent modifiers of cholestatic liver disease indicate that increased cholesterol levels correspond to higher *Pcsk9* mRNA levels in fibrotic livers. *PCSK9* variants might be associated with disease severity in PSC patients. We speculate that higher PCSK9 levels in livers of *Abcb4*-/- mice might be hepatoprotective, since they reduce the deleterious consequences of intrahepatic cholesterol accumulation.

Poster Visit Session I Basic Hepatology (Fibrogenesis, NPC, Transport) Friday, February 14, 2020, 12:30 pm – 1:15 pm, Lecture Hall P1

1.4 Shedding light on BASH: A novel experimental model of advanced liver damage

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Background & Aims Based on recent clinical studies it has been suggested to refer patients with an intermediate alcohol drinking pattern and with signs of metabolic risk, including obesity and type 2 Diabetes Mellitus, i.e. clinical features of *b*oth alcoholic and non-*a*lcoholic *s*teato*h*epatitis as *BASH*. Yet, BASH is not well described and presents a large grey area in the field of Hepatology with a huge unmet need of pre-clinical and clinical studies as well as new innovative experimental animal models.

Methods C57BL/6 female mice received 10%v/v alcohol in sweetened drinking water in combination with a Western diet (WD) up to 23 weeks (BASH model). Mice receiving either only sweetened alcohol or only WD were used as controls. Serum markers of liver damage, liver and epididymal white adipose tissue (eWAT) histology, hepatic inflammation and fibrosis progression were analysed in detail.

Results Animals fed with our novel experimental BASH model elicited a significant increase in the body-mass index (BMI) accompanied by a pronounced hypertrophy of adipocytes, as well as massive infiltration of macrophages, and robust collagen deposition in eWAT.

Moreover, progressive metabolic perturbations characterized by hypercholesterolemia and fasting hyperglycemia were found in the BASH group. In our experimental model, alcohol potentiated the effect of WD with regard to dramatic hepatomegaly, hepatocyte enlargement, profound hepatic steatosis and drastically increased levels of hepatic triglycerides. Significant liver damage was characterized by elevated plasma ALT and LDH levels, positive TUNEL staining and compensatory hepatic proliferation.

Notably, the BASH feeding also resulted in significant lobular inflammation and intrahepatic accumulation of CD45, CD11b and F4/80-positive immune cells and upregulated mRNA expression of TNFα and CCl2.

Importantly, BASH – mice exhibited advanced hepatic fibrosis, collagen accumulation, activation of hepatic stellate cells (HSC) and upregulation of Collagen IA1 and MCP-1 transcripts.

Conclusions Our novel model of BASH displays enhanced obesity, glucose intolerance, liver damage, prominent steatohepatitis and hepatic fibrosis, as well as inflammation and fibrosis in the eWAT tissue. Altogether, the BASH model perfectly mimics all histological, metabolic, transcriptomic gene signatures of human BASH and therefore it can facilitate preclinical development of therapeutic targets in the future.

1.5 Hepatocytes modify macrophage polarization by reducing the availability of active TGFβ

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The macrophage populations of the liver are heterogeneous, consisting of self-renewing tissue-resident macrophages and monocyte-derived macrophages (moMΦ) recruited from the circulation. After liver injury particularly the recruitment of moMΦ population is considered to be important for undisturbed regeneration. However, up to now it is unclear in how far an environment that is predominantly shaped by hepatocyte-derived factors influence macrophage polarization and function.

The main aim of the present study is to clarify whether a microenvironment determined by hepatocytes influences polarization of moMΦ and to identify components of the intercellular communication network that play a role in this context. Therefore, a co-culture model was established using primary

murine hepatocytes, which are co-cultivated with bone-marrow derived macrophages (BMDM) separated by a membrane that allows mediator exchange but no direct cell-cell contact. Results of these studies were further verified by *in vivo* experiments in the regenerating liver.

Evidence is provided that hepatocytes induce the up-regulation of CD 163, CD 206 and MHC class II in BMDM. This is accompanied by substantial changes of the basal mRNA expression of Arg1, IL-10, Fizz, Stab1 and IFN-beta. In addition, the inflammatory response of BMDM towards LPS is substantially altered in the presence of hepatocytes resulting in a strong increase of the expression of IL-10 and IFN-beta. Analysis of transcriptome, proteome and secretome resulted in the identification of active TGF-beta as an important signaling intermediate of the intercellular communication network. Inhibition of TGF-beta signaling in BMDM either by antibodies, inhibitors or by deletion of the TGF-beta-receptor II resulted in an upregulation of the expression of CD 163, CD 206, IL-10, Stab1 and Arg1, indicating that reduced activation of TGF-beta signaling may indeed explain the observed effects of hepatocytes on BMDM polarization. Consistent with the *in vitro* data, myeloid cell-specific deletion of the TGF-beta receptor II results in increased expression of CD 163 and CD 206 in macrophages with high expression of CD 14. In addition, the proliferative phase was prolonged during PHx liver regeneration in these animals.

The data provided indicate that hepatocytes have a strong impact on the polarization and function of BMDM and that in this context the reduction of the availability of active TGF-beta plays an important role *in vitro* and *in vivo*.

1.6 Nanoparticular bisphosphonate to selectively target and repolarize liver macrophages for anti-fibrotic treatment

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Background and Aims Bisphosphonates, e. g. Alendronate (AL), inhibit osteoclasts, a specialized population of macrophages. AL showed an antitumor effect by macrophages (re-)polarization in rat hepatocellular carcinoma [Rogers T et. al., J. Transl. Med. 2011]. However, after intravenous application, bisphosphonates are rapidly excreted by the kidneys. Therefore, we aimed to develop nontoxic nanogel particles (NHP) with covalently linked Alendronate (AL-NHP) that primarily home to the liver.

Method and Results NHP with diameters below 100 nm were synthesized by controlled-radical polymerization. AL was covalently bound to NHP to generate AL-NHP. In primary murine macrophages, AL-NHP induced a 50 % reduction of cell viability at 1 mM AL loading, equal to free AL, while NHP showed no effect. Low concentrations of AL-NHP (~30 µM AL) repolarized M2 polarized macrophages towards anti-tumorous M1 macrophages, increasing their expression of TNF-a and Interferon-g and decreasing CD 206 (mannose receptor), as determined on the transcript and protein level via qPCR and FACS analysis. AL-NHP repolarized macrophages more efficiently as equal doses of free AL, while NHP alone had no effect. After intravenous injection in healthy Balb/c mice, more than 80 % of near-infrared fluorescence labeled CS 800-AL-NHP rapidly accumulated in the liver, whereas CS 800 labeled AL was readily cleared via the kidneys. On the cellular level, CS 800-AL-NHP were effectively taken up by liver macrophages (> 90 %), endothelial cells (80 %), hepatocytes (> 90 %), portal myofibroblasts (> 90 %) and less into T-cells and NKT cells (< 10 %), as determined by FACS. In CCl4 fibrotic mice, AL-NHP (~AL 4 mg/kg or 2 mg/kg) achieved a significant and dose-dependent reduction of collagen (~60 % compared to CCl4 control mice) in the livers as determined by hydroxyproline and morphometrical collagen quantification, while free AL (~4 mg/kg) was less effective.

Conclusion We have designed AL-NHP as biocompatible carriers for the bisphosphonate AL. Nanocarrier-coupled Alendronate was almost exclusively sequestered by the liver and showed promising repolarizing effects on M2-type primary macrophages, shifting their cytokine levels towards a putative anti-fibrotic and anti-tumor M1 phenotype. CS 800-AL-NHP were efficiently taken up by liver macrophages, endothelial, fibroblastic and parenchymal cells, but less into T and NKT cells. AL-NHP exhibited in vivo a significant antifibrotic effect in CCl4 liver fibrotic mice.

1.7 Expression and Function of Four-and-a-Half LIM-domain protein 2 (FHL 2) in Hepatic Fibrosis

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The four-and-a-half LIM-domain protein 2 (FHL 2) is a scaffolding protein modulating multiple signal transduction pathways in a tissue- and cell context- specific manner. Some studies revealed a role of FHL 2 in fibrotic diseases of different organs as well as in different types of cancer including hepatocellular carcinoma.

The aim of this study was to investigate the expression and function of FHL 2 in hepatic fibrosis.

Methods and Results RT-qPCR and Western Blot analysis revealed that FHL 2 mRNA and protein expression were significantly increased in different murine models of liver fibrosis. Furthermore, we found significantly elevated FHL 2 expression in liver tissues of patients with chronic hepatitis C infection (HCV) and advanced fibrosis (staging 3 and 4) compared with HCV-patients with lower fibrosis stages. Furthermore, expression levels of FHL 2 correlated significantly with the expression of collagen type I and alpha-smooth muscle actin (alpha-sma) in diseased human liver tissues. Alpha-sma is a marker for the activation of hepatic stellate cells (HSC), the key event of hepatic fibrosis. In line with this, immunohistochemistry showed a strong alpha-sma immunosignal in fibrotic septa of cirrhotic liver tissues. Furthermore, FHL 2 increased significantly during the in vitro activation of primary HSCs. To further assess the role of FHL 2 in liver fibrosis, we applied the bile duct ligation (BDL) model to FHL 2-deficient (FHL 2-ko) and wildtype (wt) mice. BDL caused a more severe portal and parenchymal inflammation as well as extended portal fibrosis in FHL 2-ko compared to wt mice. In line with this, BDL-induced pro-inflammatory (TNF, IL-1, MCP-1, ICAM-1) and pro-fibrogenic (alpha-sma, TGF-beta, Collagen Type I) gene expression was significantly higher in livers of FHL 2-ko compared to wt mice.

Summary and Conclusion The marked upregulation of FHL 2 in hepatic fibrosis exhibits anti-inflammatory and anti-fibrogenic effects. These data indicate FHL 2 as potential prognostic marker and therapeutic target for hepatic fibrosis in patients with chronic liver diseases.

1.8 Genetic or pharmacological inhibition of cyclin-dependent kinase 2 in Hepatic Stellate Cells acts anti-fibrotic

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Question Liver fibrosis is a wound healing process in response to chronic liver injury, characterized by regenerative proliferation of hepatocytes and the accumulation of extracellular collagen produced by Hepatic Stellate Cells (HSCs). This process involves cell cycle re-entry and proliferation of the normally quiescent HSCs controlled by cyclins and associated cyclin-dependent kinases (Cdks). Cdk2 mediates the entry and progression through S-phase (*i.e.* DNA synthesis) in complex with E-and A-type cyclins. We have recently demonstrated that Cyclin E1 is essential for liver fibrogenesis in mice. However, so far it was not known, if this function was dependent on Cdk2. Thus, in this study we tested the requirement for Cdk2 and related kinases in HSCs.

Methods We generated conditional, HSC-specific Cdk2 knockout mice using transgenic cre-expression under control of the L-rat promoter. These mice were challenged with CCl4 for 6 weeks and subsequently investigated for liver fibrosis. For pharmacological *in vitro* analyses we used the pan-Cdk inhibitor CR8 with highest specificity against Cdk2 and Cdk1. This drug was tested for anti-fibrotic and cytotoxic properties on human (LX-2) and murine (GRX) HSC cell lines as well as on primary murine HSCs and hepatocytes.

Results Genetic ablation of Cdk2 specifically in HSCs significantly reduced collagen accumulation in the liver after CCl4 treatment. This suggests that cell cycle re-activation of naïve HSCs *in vivo* requires functional Cdk2 presumably in complex with Cyclin E1. In order to translate these findings into a preclinical therapeutic model, we challenged human LX-2 and murine GRX cells with the pan-Cdk inhibitor CR8. CR8 treatment substantially reduced Cdk kinase activity in both cell lines. In addition CR8 inhibited proliferation, survival and pro-fibrotic activation in both LX-2 and GRX cells, and also triggered DNA damage and cell cycle arrest. Importantly, we identified effective CR8 dosages mediating anti-fibrotic effects in primary murine HSCs without affecting cell cycle activity and survival in primary hepatocytes.

Conclusion The pro-fibrotic properties of HSCs depend on functional Cdk2 *in vivo*. In addition, pharmacological pan-Cdk inhibition reduced the fibrogenic functions of HSC lines and primary HSCs without affecting the regenerative capacity of hepatocytes *in vitro*. We thus conclude that HSC-specific inhibition of Cdk2 could be a novel therapeutic approach to treat liver fibrosis.

1.9 Loss of hepatic Mboat7 leads to liver fibrosis in an inflammation-independent manner

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Background & Aims Human association studies have identified association of the rs641738 C>T variant in membrane-bound O-acyltransferase domain containing 7 (*MBOAT7*) with non-alcoholic fatty liver disease (NAFLD). We aim to better understand the mechanism by which the rs641738 variant contributes to the pathogenesis of NAFLD.

Methods Mice with hepatocyte-specific deletion of Mboat7 (Mboat7Δhep) were generated and livers characterized by histology, flow cytometry, qPCR, RNA sequencing and lipidomics. We analyzed the association of the rs641738 genotype with liver inflammation and fibrosis in 361 NAFLD patients and obtained genotype-specific liver lipidomes from 336 human biopsies.

Results Allelic imbalance analysis of heterozygous human liver samples pointed to lower expression of the MBOAT7 transcript on the rs641738T haplotype. Mboat7Δhep mice showed spontaneous steatosis characterized by increased hepatic cholesterol ester content after 10 weeks on a chow diet. After 6 weeks on a high fat, methionine-low, choline-deficient diet, mice developed increased hepatic fibrosis as measured by picrosirus staining ($p < 0.05$) and hydroxyproline content ($p < 0.05$) while the inflammatory cell populations and inflammatory mediators were not changed. In a human biopsied NAFLD cohort, MBOAT7 rs641738T was associated with fibrosis ($p = 0.004$) independent of the presence of histological inflammation. Liver lipidomes of Mboat7Δhep mice and human rs641738TT carriers with fibrosis showed increased total lysophosphatidylinositol (LPI) levels. The remodeling of LPI and phosphatidylinositol subspecies patterns in Mboat7Δhep livers and humans rs641738 TT carriers were similar.

Conclusion Mboat7 deficiency in mice and human points to an inflammation-independent pathway to liver fibrosis, that may be mediated by lipid signalling and represents a potentially targetable treatment option in NAFLD.

1.10 Improved Hepatogenic Differentiation of Human Adipose Tissue-derived MSCs Using a Silicone-based Cell Culture System

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Conventional two-dimensional as well as established three-dimensional cell culture systems exhibit a high modulus of elasticity, yet, without representing the stiffness of human organs. Previously, we established a protocol for the culture of primary rat hepatocytes on silicone, which sustained their physiological functions. In terms of the development of human-based tests, the aim of this study was to improve hepatogenic differentiation of human adipose tissue-derived mesenchymal stromal cells (hAT-MSC) on fibronectin-coated 3D silicone scaffolds with low stiffness (E-modulus 250 kPA) mimicking the stiffness of human liver including surface modifications to increase hydrophilic properties.

hAT-MSCs were cultured on 3D silicone scaffolds. Hepatogenic differentiation was initiated by the 2 step protocol. The capability of the hepatocyte-like cells to store glycogen, to synthesize urea and albumin as well as the enzyme activities of CYP1A1 and Cyp2B1/2 were analysed and compared to the conventional 2D cell culture and hepatogenic differentiation on polystyrene (E-modulus 103 MPa).

hAT-MSC attached to the 3D silicone scaffolds and formed cell clusters. 10 days after starting the hepatogenic differentiation until the end of the culture on day 24, the cells on 3D silicone scaffolds displayed more intensive PAS staining for glycogen as compared to the cells on 2D cultures indicating improved glycogen storage. The urea synthesis rate [nmol/10.000 cells/24 h] of these cells was significant higher as compared to cells on 2D culture throughout the culture period, though declining with ongoing culture; 10 d: 2D vs. 3D 0.05 ± 0.01 vs. 1.68 ± 0.05 and 24 d: 0.03 ± 0.007 vs. 0.4 ± 0.04, respectively. The albumin synthesis rate [pg/10.000 cells/24 h] was significant higher in cells differentiated for 24 d as compared to undifferentiated cells: 48.5 ± 11 vs. 7.4 ± 1.9. Enzyme activity of CYP1A1 after 24 d was 3-fold higher in cells differentiated on 3D silicone scaffolds as compared to cells cultivated on 2D and the enzyme activity of CYP2B1/2 was 12-fold higher.

With these results, medically approved silicone treated with plasma was identified to improve hepatogenic differentiation of hAT-MSC, likely by providing the appropriate three-dimensional growth conditions. Thus, the silicone scaffolds represent an important tool for the development of cell cultures featuring organ-like stiffness and improving physiological functions of hepatogenic differentiated MSC.

Thieme

1.11 Hepatic iron overload in alcoholic liver disease: The role of sinusoidal endothelial cells in iron sensing

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Background and Aims So far, hepatic iron overload in patients with alcoholic liver disease is poorly understood. Hepcidin, the master switch of systemic iron homeostasis and is regulated by the BMP signaling pathway. Recent data showed that liver sinusoidal endothelial cells (LSECs) express the highest amount of BMP6 among different hepatic cell types and are able to regulate iron homeostasis *in vivo*. However, the exact mechanisms, how iron levels are sensed by ECs and how BMP signaling is involved in the regulation of systemic iron metabolism as well as which cells are involved is still not completely known. The aim of this study is to investigate the crosstalk between LSECs and hepatocytes in regulating iron metabolism.

Methods Huh7 cells (hepatocytes) and HUVECs (Human Umbilical Vein Endothelial Cells) were cultured alone and treated with holo-transferrin (Holo-Tf), ferric ammonium citrate (FAC) and salicylaldehyde isonicotinoyl hydrazine (SIH) as an iron chelator under hypoxic condition (1% O2) for 24 hours. Next, co-cultures of HUVECs and Huh7 cells were established by using the supernatant of HUVECs to incubate Huh7 cells. Hepcidin, BMP6, TFR1 were assessed by qRT-PCR or Western blot. Meanwhile the Bmp6 concentrations in medium were detected by ELISA. To investigate the function of BMP6 in the co-culture system, we also neutralized BMP6 with monoclonal antibody in the supernatant of HUVECs and incubated Huh7 cells with the neutralized supernatant.

Results Ferric iron significantly led HUVECs to secret more Bmp6 into the culturing supernatant under 1% O2, whereas no effect on Huh7 cells was detected. Expression of hepcidin in Huh7 cells was remarkably increased after incubation with the supernatant of HUVECs cultured under 1% O2 for 24 h regardless of treatments with iron if compared to fresh medium control group. In the meantime, hepcidin expression in Huh7 cells induced by the supernatant of HUVECs could be remarkably blocked by using neutralizing BMP6 antibody.

Conclusion Endothelial cells are able to sense iron changes (iron supplementation or chelation) and the production of endothelial cells can induce the hepcidin expression in hepatocytes. BMP6 played an essential role in hepcidin expression in hepatocytes, whereas the hepcidin expression quantity is not related to the BMP6 concentration in the surroundings. Further explorations are necessary to better understand the crosstalk between endothelial cells and hepatocytes on iron regulation.

1.12 The role of PNPLA3 and MBOAT7 during alcohol detoxification: Different mechanisms for fibrosis development

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Background and Aims In genome wide association studies PNPLA3 and MBOAT7 were identified as important risk genes for the development of alcoholic cirrhosis, however, their functions and molecular mechanisms are still poorly understood. We here present first data on the role of PNPLA3 and MBAOT7 genotypes on liver stiffness (LS), steatosis (CAP) and inflammation during alcohol withdrawal.

Method 763 patients with ALD which were hospitalized for alcohol withdrawal at Salem Medical Center between 2007 and 2018 were genotyped for PNPLA3 s738409 and MBOAT7 rs626283 polymorphisms. All patients had routine laboratory, abdominal ultrasound and a transient elastography measurement (FibroScan) at admission. In 512 patients, data after 6.3 days of alcohol withdrawal was available.

Results 71% of the patients were male, median age was 52 years, median BMI was 24.7 kg/m2 and median alcohol consumption was 163 g/day. At admission, no difference between the genotypes of PNPLA3 and MBOAT7 was seen regarding age, BMI, gender, alcohol consumption or transaminase levels. Significant differences were observed for PNPLA3 and MBOAT7 during alcohol detoxification. While MBOAT7 was associated with higher LS, no differences were observed between genotypes upon alcohol detoxification. In contrast, PNPLA3 caused clearly a delayed resolution of LS during withdrawal of alcohol due to inflammation. This could be recapitulated when looking at serum markers of liver inflammation. In a sub-analysis of n = 108 liver biopsies, inflammation was highly associated with PNPLA3 but not MBOAT7. More interestingly, PNPLA3 was associated with higher steatosis (CAP) although it resolved faster upon detoxification. No effect at all was seen for MBOAT7 on steatosis. A multivariate model confirmed that PNPLA3 was associated with steatosis and inflammation but not fibrosis. MBOAT7 was only associated with fibrosis/cirrhosis but not inflammation or steatosis.

Conclusion These first genotype data on a "human alcohol knock-out" intervention underscore important differences between PNPLA3 and MBOAT7. PNPLA3 seems to primarily drive fibrosis through inflammation and our data on CAP suggest an enhanced fat metabolism. In contrast, MBOAT7 seems to have a direct effect on fibrosis signaling and is neither associated with steatosis nor inflammation. Finally, alcohol detoxification could be a novel interventional approach to further dissect metabolic mechanisms and their associations with genotypes.

1.13 Hippo pathway effectors YAP and TAZ are opponents in the regulation of hepatic fibrosis

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Question Paracrine cross-talk between liver-resident cells affects the development and progression of fibrosis. For example, overexpression of the transcriptional co-activator *yes-associated protein* (YAP) in hepatocytes supports deposition of extracellular material of hepatic stellate cells. We therefore asked, if silencing of hepatocellular YAP and its paralogue *WW domain containing transcription regulator 1* (WWTR1; syn: TAZ) may represent a reasonable approach to reduce liver fibrosis.

Methods As model, we systematically characterized mice with hepatocyte-specific inactivation of YAP (YAPKO), TAZ (TAZKO) as well as YAP/TAZ (YAPKO/TAZKO) and induced liver fibrosis by intraperitoneal injection of carbon tetrachloride (CCl4; 2x/week for 6 weeks).

Results Serum derived from unchallenged YAPKO and YAPKO/TAZKO, but not from wildtype and TAZKO animals, showed markedly higher levels of liver damage markers (ALT: up to 792 U/L; AST: up to 867 U/L). Interestingly, automated whole slide analysis after Sirius red staining revealed the development of severe fibrosis in YAPKO livers, which was less pronounced in animals with combined YAP/TAZ silencing. Four weeks after the last CCl4 injection, ALT and AST of YAPKO and YAPKO/TAZKO mice were still elevated, while in wildtype and TAZKO mice both liver damage makers normalized. Importantly, compared to YAPKO mice, AST/ALT levels were significantly lower in YAPKO/TAZKO animals. Livers derived from CCl4-treated YAPKO mice showed significantly elevated liver fibrosis compared to untreated YAPKO animals, while the simultaneous silencing of YAP/TAZ partly abolished this phenotype (automated picture analysis and Ishak score). Histologically, CCl4 treatment caused clear bridging fibrous septa in wildtype and TAZKO mice. In contrast, YAP-

deficiency led to the formation of profound diffuse septal liver fibrosis. Again, this phenotype was dampened in samples derived from YAPKO/TAZKO mice.

Conclusions These data illustrate that hepatocellular YAP activity is key for the maintenance of liver organization and extracellular material deposition. Its perturbation does not present a reasonable approach to block liver fibrosis since YAP-deficiency is sufficient to cause fibrosis. In this regard, YAP and TAZ have different functions in fibrosis. While YAP-deficiency aggravates fibrogenesis, TAZ-deficiency alone plays as protective and dampens the effects observed after YAP-silencing.

1.14 Common genetic variant c.711A>T in the hepatobiliary phospholipid translocator *ABCB4* as risk factor for liver fibrosis

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Question Chronic liver diseases (CLD) result in liver fibrosis. Recently it was demonstrated (Serra-Burriel et al. *J Hepatol* 2019) that a transient elastography (TE) threshold of 9.1 kPa has the best accuracy for the prediction of relevant liver fibrosis (i.e., stages ≥ F2). Previously we showed that the common *PNPLA3* (adiponutrin) p.I148 M polymorphism is associated with liver cirrhosis and that carriers of the procholestatic *ABCB4* c.711T allele, who are also positive for the *PNPLA3* variant, might be at risk of progressive liver injury. Here, we investigate the association between this *ABCB4* polymorphism and liver stiffness as well as the influence of *ABCB4* and *PNPLA3* on fibrosis in a mouse model of chronic cholestasis.

Methods Prospectively we recruited 712 patients (278 women, age 50 ± 13 years, BMI 24.4 ± 4.3 kg/m2) with CLD. Liver stiffness was measured non-invasively using TE. The *ABCB4* c.711A>T polymorphism was genotyped by a PCR-based assay. *Pnpla3* expression as well as liver injury were analysed in the *Abcb4*-/- mice and controls animals.

Results Median liver stiffness was 6.7 kPa and 226 individuals (31.7%) presented with TE ≥ 9.1 kPa. The minor allele frequency of the *ABCB4* c.711 was 0.18 in the human cohort. The *ABCB4* variant was significantly (P = 0.02, OR = 1.33) associated with liver stiffness ≥ 9.1 kPa. In a multivariate model including non-genetic profibrogenic factors, the *ABCB4* variant (P = 0.05, OR = 1.43) as well as BMI (P = 0.04, OR = 1.04) and age (P < 0.01, OR = 1.02) all proved to be independent risk factors for fibrosis stage ≥ F2. Moreover, *Abcb4* deficient mice showed enhanced liver injury (reflected by increased *Col1a2* expression and elevated collagen contents), which was coupled with significantly (P < 0.05) lower expression of *Pnpla3* as compared to wild-type mice.

Conclusions The procholestatic *ABCB4* c.711T allele might represent a common genetic risk factor for clinically relevant liver fibrosis. Lower expression of adiponutrin in fibrotic livers of the *Abcb4*-/- mice alludes to the interaction between phospholipid content and *PNPLA3* expression in progressive liver injury. Our results suggest that the *Abcb4*-/- might serve as the animal model to further investigate the role of *PNPLA3* in liver fibrosis.

1.15 RAGE signaling in liver progenitor cells affects fibrosis upon liver injury

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Persistent liver injury results in abundant cell death and activation of liver progenitor cells (LPCs). The receptor for advanced glycation end products (RAGE) signaling axis is often associated with chronic inflammation-associated tissue damage and plays an essential role in modulating the tumor microenvironment. It functions as a key regulator in LPC activation mediated by damage-associated molecular pattern (DAMP) molecules, such as HMGB1 and S100 proteins that are released by immune and necrotic cells.

In this study, we aim to delineate the functional role and underlying mechanism of RAGE activity in LPC activation in response to inflammation-associated liver injury. R26TomHnf1β-CreER transgenic mice were crossed with RAGE flox/flox (RAGEfl/fl) mice to generate tamoxifen-inducible LPC-specific RAGE knockout mice (RAGEΔLPC). They were exposed to a choline-deficient ethionine-supplemented (CDE) diet for three weeks to induce liver damage. Although RageWT and RageΔLPC mice showed comparable levels of liver injury, inflammation and steatosis upon CDE treatment, ablation of RAGE in LPCs strongly impairs LPC expanding and migratory capacities, which are accompanied by reduction of bridging fibrosis, suggesting that RAGE signaling in LPCs is a mediator of liver fibrosis.

Primary LPCs were isolated from CDE-treated Ragefl/fl C57BL/6 mouse and cultured *in vitro*. A Rage knockout cell line was established by transient transfection of a Cre recombinase-carrying plasmid. LPCs were stimulated with supernatants from necrotic hepatocytes (necrotic medium) followed by RNA-Seq to identify downstream targets of RAGE-dependent pathways. Stress response, inflammatory and pro-fibrotic pathways, such as TNF, ErbB, TGF-Beta and NF-kB pathways were induced in LPCs upon treatment with necrotic medium. Most interestingly, Hippo signaling, PI3K-Akt signaling and leukocyte transepithelial migration-associated pathways were found to be RAGE-dependent. Moreover, clusters of stem cell renewal-related genes, such as EpCam, Nf2, Bmp4 and Notch1 were deregulated upon ablation of RAGE. Consistent with the RNA-seq results, we demonstrated that ablation of Rage attenuates LPCs organoid-forming ability.

Our recent results implied that RAGE regulates stemness properties of LPCs and is required for LPCs activation in supporting fibrogenesis. Taken together, our data provide a potential mechanistic insight on the role of RAGE in LPCs in association with fibrosis upon chronic liver injury.

1.16 Fibrocytes contribute to TAA-induced liver fibrosis in mice

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Background Bone marrow-derived fibrocytes represent a unique cell type, sharing features of both mesenchymal and hematopoietic cells. They comprise ~0.5% of peripheral blood leucocytes and have been implicated in fibrotic diseases of the kidney, lung, heart, and liver. Fibrocytes were shown to specifically infiltrate the injured liver and participate in the deposition of extracellular matrix components. Moreover, fibrocytes hold the potential to regulate fibrogenesis via the secretion of TGF-β, MMPs, TNF-α, IL-1β, and other inflammatory cytokines. Their relevance to hepatic fibrosis, however, remains unknown. We aimed to study the effect of a fibrocyte depletion during fibrogenesis in a mouse model of chronic-toxic liver fibrosis.

Methods Fibrocytes were depleted utilizing a suicide gene strategy. Bone marrow of transgene C57BL/6J mice, expressing a herpes simplex virus thymidine kinase (HSV-TK) under the control of a collagen I promotor, was transplanted into lethally irradiated mice of the same genetic background (n = 16 per group, mice of the control group received wild-type bone marrow). Thioacetamide (TAA) and Valganciclovir, which is metabolized into toxic compounds by the HSV-TK, were administered via drinking water for 18 weeks to induce fibrosis and specifically deplete bone marrow-derived fibrocytes.

Results TAA-administration induced a marked perilobular fibrosis. RNA *in situ* hybridization visualized fibrocytes in liver samples of the control group and proved the suicide gene strategy's success. The depletion of fibrocytes lead to a significantly reduced amount of fibrillar collagens, indicated by decreased hepatic hydroxyproline content (-7.8%; 95% CI: 0.7 – 14.8%; p= 0.033). The expression and distribution of α-SMA was unchanged in western blot, immunohistochemistry, and RT-qPCR in result of the fibrocyte ablation. High-throughput analyses of a panel of MMPs and inflammatory mediators yielded no relevant regulations. Yet lower serum ALT levels (-20.9%; 95% CI: 0.4 – 36.9%; p= 0.049) show a mitigation of liver-specific cellular damage.

Conclusion Our model enabled the study of fibrocytes in a complex *in vivo* setting. Fibrocytes functionally contribute to TAA-induced liver fibrosis, as our results suggest, independent of the activation or proliferation of myofibroblasts.

1.17 Hydrophobe Gallensäuren wirken durch PI3K-abhängige Proliferation von Sternzellen pro-fibrotisch

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Question Chronisch cholestatische Lebererkrankungen führen im Verlauf regelhaft zur Leberfibrose. Es wird angenommen, dass hydrophobe Gallensäuren (GS) dabei pro-fibrotisch wirken. Die Mechanismen der Cholestase-induzierten Leberfibrose sind jedoch kaum bekannt. GS-induzierte pro-proliferative Signale in hepatischen Sternzellen (HSC) wurden als möglicher Mechanismus beschrieben. Ob diese Proliferation auch zur Anreicherung von HSC und vermehrter Kollagenanreicherung führt, wurde bisher nicht untersucht und zugrundeliegende Signalwege sind unvollständig charakterisiert. In dieser Arbeit soll daher der pro-fibrotische Effekt von GS in murinen Sternzellen (mHSC) näher charakterisiert und zugrundeliegende Signalwege exploriert werden.

Methods mHSC wurden aus weiblichen FVB-Mäusen isoliert, aufgereinigt und mit GS in An- und Abwesenheit von Inhibitoren der PI3-Kinase (PI3K) behandelt. Mittels BrdU-Assay wurde die Zellproliferation, mikroskopisch die Zellzahl und in weiteren Versuchen die DNA-Menge als Surrogat der Zellzahl erfasst. Die Kollagenablagerung wurde mittels Siriusrot-Färbung quantifiziert. Die Aktivierung von Signaltransduktionswegen wurde mittels Western Blot evaluiert. Die humane HSC-Zelllinie LX2 wurde mit TGF-β (10 ng/ml) stimuliert und die Aktivierung durch Quantifizierung der αSMA erfasst.

Results Die im Menschen quantitativ bedeutsamste hydrophobe GS Chenodeoxycholsäure (CDCA), nicht aber Cholsäure (CA) oder die hydrophile Ursodesoxycholsäure (UDCA), führten zu einer gesteigerten Proliferation der mHSC. Dies war mit einer Zunahme der Zellzahl nach 14 Tagen Kultur assoziiert. Die DNA-Menge stieg dosisabhängig auf das 1,90 ± 0,19-fache (250 µM CDCA, MW ± SD, $p < 0,05$). Die Akkumulation von Sternzellen führte zur erhöhten Ablagerung von Kollagen. Dies war mit einer dosisabhängig erhöhten Phosphorylierung der Proteinkinase B (PKB) sowohl in der Kurz- (2 – 4 Stunden) als auch Langzeitstimulation (7 – 10 Tage) assoziiert. Hemmung der PI3K mittels LY294002 (10 µM) blockierte sowohl die CDCA-induzierte PKB-Phosphorylierung als auch die Zunahme der Zellmasse. Inhibition der PI3K unterdrückte auch in humanen LX2-Zellen deren TGF-β-abhängige Aktivierung.

Conclusions Hydrophobe Gallensäuren führen zur Anreicherung von Sternzellen und gesteigerten Kollagenablagerung *in vitro*. Diese pro-fibrotische Wirkung wird möglicherweise durch PI3K-abhängige Signale vermittelt. Dieser Signalweg scheint auch für die Aktivierung humaner Sternzellen relevant.

1.18 Macrophage-specific Pla2G6 deficiency modulates bone marrow Ly6C levels and APAP liver injury in a sex-dependent manner

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Group VIA iPLA2 (iPLA2b) or PLA2G6 catalyzes hydrolysis of phospholipids at the sn-2 position, releasing a fatty acid and a lysophospholipid which has been shown to mediate neutrophil adhesion and monocyte migration in immune cells. Moreover, PLA2G6 single nucleotide polymorphism is associated with serum C-reactive protein suggesting PLA2G6 role in inflammation. Therefore, we aim to investigate whether Pla2G6 deficiency in macrophages could modulate liver injury by altering differentiation programs of lymphocyte antigen 6 complex (Ly6C) in the bone marrow.

Methods Macrophage-specific (lysMCre) Pla2G6 KO mice with exon 6 – 8 deletion were generated. Bone marrow-derived macrophages (BMDM) from control and KO mice were prepared. Acute liver injury was induced by intraperitoneal injection of acetaminophen (APAP 300 mg/kg), and mice were killed 24 h later. Liver injury and gene expression analyses were assessed. BMDM phospholipids were measured by LC-MS. CD45+CD11b+CD115+Ly6G-Ly6C+ monocytes were measured by FACS.

Results BMDM of KO mice showed the absence of Pla2G6 protein and mRNA. Livers of KO mice still showed Pla2G6 protein expression indicating specific deletion in macrophages. By LC-MS analysis, BMDM from KO mice showed a significant decrease in monounsaturated lysophosphatidylcholine (LPC) and 18:2 LPC, and an increase of phosphatidylinositol (PI) 40:5 and phosphatidylserine (PS) 40:5. Upon APAP treatment, male KO mice showed a significant increase of AST, and a trend increase of ALT, LDH, and caspase3 activity. BMDM and livers of male KO mice respectively showed increased IL-6 mRNA and protein expression. The analyses of Ly6C mRNA expression and % ly6C+ monocytes by FACS showed no difference between male control and KO. On the other hand, female KO mice showed no worsening liver injury. However BMDM of female mutants showed a significant increase of ly6C mRNA expression, a trend increase of the M2 markers, Retnla and Chi3l3. Consistently, FACS results of BMDM showed a significant increase of % ly6C+ monocytes in these female KO mice.

Conclusions Pla2G6 has specificity in the hydrolysis of PI and PS in BMDM. Male KO BMDM showed no alteration of ly6C while female KO BMDM showed an increase of ly6C. As ly6C is thought to be involved in inflammation and resolution and that male KO mice showing worsen liver injury than female KO mice, hence deficiency of Pla2G6 in macrophages may modulate acute liver injury in a ly6C- and sex-dependent manner.

1.19 Pulmonary arterial hypertension leads to liver fibrosis in the SU5416/hypoxia model in rats

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Questions Patients with pulmonary arterial hypertension (PAH) may develop liver fibrosis/cirrhosis and portal hypertension over time (Reverter, Seijo, & Bosch, 2012). The underlying mechanisms for this are largely unknown. We and others have shown, that the SU5416/hypoxia (SuHx) rat model is a model of severe PAH, whether these animals also develop liver fibrosis is unknown.

Methods Six to eight-week old male Sprague-Dawley rats were purchased from Charles River and divided into three different treatment groups: (A) normoxia (ConNx), (B) ConHx [injected once s.c. with vehicle (DMSO), then exposed to chronic hypoxia (FiO2 0.1) for 3 weeks, followed by a 6-week period in room air (FiO2 0.21)]; (C) SuHx [injected with the VEGFR2 inhibitor SU5416 Σ, 20 mg/kg per dose s.c. dissolved in DMSO and subsequently exposed to chronic hypoxia (3 weeks), followed by 6 weeks of room air]. At the end of treatment animals were sacrificed and livers were harvested for subsequent analysis. H&E, Masson"s trichrome and Picrosirius red stainings were performed according to routine protocols. RNA for qPCR and transcriptome analysis was isolated using TRIzol reagent.

Results Picrosirius red staining showed no difference in liver fibrosis between rats in the normoxia (ConNx) and hypoxia (ConHx) groups (0.88-fold induction, $p = 0.9734$). Rats in the SuHx treatment arm developed significant PAH (Right ventricular systolic pressure (RSVP) ConNx vs. SuHx, 29 vs. 91 mm Hg, $p < 0.0001$) and also displayed significant liver fibrosis (1.60-fold induction compared to ConNx, $p < 0.01$). QPCR analysis confirmed induction of fibrogenic genes Timp1 (2.12-fold induction, $p < 0.05$) and col1a1 (3.13-fold induction, $p < 0.01$) in the SuHx rats. H&E- and trichrome staining also showed immune cell infiltration in the livers of the SuHx treated animals. RNA-sequencing of whole liver tissue revealed an upregulation of the inflammatory response, T-cell activation, cell growth and fibroblast proliferation in animals with PAH and liver fibrosis.

Conclusions Pulmonary arterial hypertension leads to liver fibrosis in the SU5416/hypoxia model in rats. Patients with PAH should therefore also be screened for signs of liver fibrosis.

1.20 WISP1: A novel extracellular matrix remodeling protein in liver fibrosis

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Liver diseases are a global burden and a better understanding of factors controlling disease progression is required. In the last decade, great knowledge has been developed regarding the biological role of the extracellular matrix composition. Alterations in this dynamic structure can either facilitate or impair the repairmen of damaged liver tissue. That is why, novel components like matricellular proteins from the CCN family, have emerged as new targets in liver pathophysiology. These highly conserved secreted proteins specifically interact with and signal through various extracellular partners, like integrins, which enable them to play crucial roles in various processes including development, wound healing and diseases such as cancer and fibrosis.

We have discovered that WISP1 (Wnt-induced secreted protein-1) also named CCN4, is induced upon CCl4-induced liver damage and may play an important role in the remodeling process of the extracellular matrix. Thus, we aim to study WISP1 cell source and its influence in cell migration and fibrosis model in WISP1 KO mice.

Isolation of individual liver cell types and quantification of WISP1 expression and secretion showed a higher mRNA expression and TGF-β-induced secretion of WISP1 in non-parenchymal cells, especially in stellate cells but also in liver sinusoidal endothelial cells compared to hepatocytes. Furthermore, WISP1 facilitates the migration of isolated mouse hepatic stellate cells through collagen lattices, suggesting the interaction of WISP1 with one of the main components of the extracellular matrix. Additionally, gene expression analysis and Sirius Red staining showed differences in the development of CCl4-induced fibrosis between WISP1 wild type and knockout mice. Upregulation of collagen type I and α-SMA is reduced in WISP1 KO mice and less breaching of the collagen deposition is also observed.

In conclusion, WISP1 is mainly expressed and secreted by stellate cells which may influence their migration upon chronic liver injury.

1.21 Absence of JNK activity in hepatocytes exacerbates liver injury and fibrosis during chronic cholestasis in mice

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Background The c-Jun NH2-terminal kinase (JNK) activation is required for cholestatic liver injury-induced fibrogenesis. Global JNK1 or JNK2 functions have been thoroughly addressed. Moreover, we showed that JNK1 function in hepatic stellate cells (HSCs), but not in hepatocytes mediates transactivation of HSCs during murine liver fibrosis. Here, we tested the hypothesis that JNK1 and JNK2 together in hepatocytes work to confer protection during cholestatic liver injury-induced liver fibrosis.

Methods The relevance of JNK in human and experimental cholestatic liver disease was tested. Additionally, Jnk1/2$^{f/f}$ (WT) and Jnk1/2$^{\Delta hepa}$ (hepatocyte-specific deletion of JNK1 and JNK2) mice were subjected to bile duct ligation (BDL) for 28 days. *Mdr2* knockout mice were also used. Moreover, microarray analysis was performed.

Results Activation of JNK is characteristic in human (primary biliary cholangitis, PBC and primary sclerosing cholangitis, PSC) and in murine cholestasis (Mdr2$^{-/-}$ and BDL). Serum markers of hepatic damage – liver transaminases – and liver histology revealed increased cell death, hepatic fibrogenesis, oxidative stress and inflammation in Jnk1/2$^{\Delta hepa}$ mice compared to WT, 28-days after BDL. Furthermore, microarray analysis indicated protection of the mucosal epithelium since Mucin and Trefoil family members were strongly upregulated in absence of hepatocytic JNK1/2.

Conclusion Combined function of JNK1 and JNK2 in hepatocytes protects against the development of cholestatic liver disease.

1.22 Expanded primary human liver sinusoidal endothelial cells as a tool for complex hepatotoxicity studies

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Liver sinusoidal endothelial cells (LSECs) are highly specialized endothelial cells lining the walls of hepatic sinusoids. Their key roles include liver regeneration, the transfer of substrates between blood and liver parenchyma, rapid internalization of blood-borne macromolecules as well as immune tolerance. Despite their substantial contribution to liver homeostasis, LSECs are often overlooked during hepatotoxicity assays due to insufficient cell yields after isolation and a restricted proliferation capacity *in vitro*.

To address these issues, we expanded primary LSECs derived from 3 donors by lentiviral transduction with proliferation inducing genes (upcyte® technology). Transduced LSECs performed 28 – 45 population doublings in a donor-dependent manner until senescence occurred. Generated upcyte LSECs expressed typical endothelial cell markers (CD 31, von Willebrand factor) and showed marked binding of UEA-1 (Ulex Europaeus Agglutinin I). In addition, we found expression of several LSEC-associated receptors including MMR (mannose receptor), LYVE-1 (lymphatic vessel endothelial hyaluronan receptor 1) and FCGR2B (inhibitory receptor for the Fc region of immunoglobulin gamma). Expanded LSECs further revealed marked uptake of macromolecule ligands (ovalbumin, acetylated low density lipoprotein) and were capable of tube formation when cultured in Matrigel.

Since LSECs are involved in drug-induced liver injury, we challenged the cells with several hepatotoxic model compounds. Interestingly, upcyte LSECs were more susceptible to e. g. acetaminophen and imipramine-induced toxicity when compared to upcyte hepatocytes, indicating that these cells constitute a useful tool to complement hepatotoxicity evaluation.

Taken together, our data suggest that upcyte LSECs combine many characteristics of primary LSECs with the advantage of an extended lifespan, facilitating their use in hepatotoxicity assays under reproducible and standardized conditions. Future applications include e. g. in vitro uptake assays of ADCs (antibody drug conjugates) or triggering the hepatic immune response via the inclusion of T-cells and their antigen presenting capabilities to the LSECs.

1.23 Extracellular vesicles from steatotic hepatocytes influence stellate cells in liver fibrosis

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Background Liver fibrosis, caused by fatty degeneration, is a major cause of liver failure, and is also associated with higher levels of hepatocellular carcinoma. Although the pathophysiologic process leading to liver fibrosis is not completely clarified, stellate cells appear to play an important role. In this study, we have investigated a possible interplay between hepatocytes under normal and steatotic conditions. Our hypothesis was that extracellular vesicles (EV) isolated from hepatocytes could influence the behavior and phenotype of stellate cells.

Methods By high speed centrifugation, EV were isolated from the conditioned media of hepatocellular carcinoma cell line HepG2, under baseline conditions (C-EV) or after induction of steatosis by a mixture of linoleic and oleic acid (FA-EV). The EV were incubated with the stellate TWNT4 cell line and expression of matrix remodeling markers was determined by qPCR and western blotting. Migration of TWNT4 cells was investigated using Boyden chambers. Determination of the cell content was done by mass spectrometry.

Results The migration of TWNT4 cells towards sera obtained from patients with clinically diagnosed NASH was increased compared to sera from matched controls. Induction of steatosis in HEPG2 cells resulted in increased EV release compared to basal conditions. Chemotactic migration of TWNT4 cells was increased towards both C-EV and FA-EV. TWNT4 cells were incubated with the EV obtained from resting and steatotic HEPG2 cells. Interestingly, when TWNT4 cells were incubated with FA-EV, chemotaxis towards CCL 5 was reduced. Measurement of matrix remodeling markers after treatment revealed that the expression of the collagen type 1a1 gene (COL 1A1) did not change after EV-treatment, yet RNA levels of matrix metalloprotease 2 (MMP2) were reduced. Likewise, the expression of the markers MMP14, tissue inhibitor of MMP (TIMP1) and platelet-derived growth factor (PDGF) was decreased, albeit only after treatment with FA-EV. Differentiation of stellate cells to myofibroblasts is known to drive fibrosis. Incubation of TWNT4 cells with FA-EV led to a decreased expression of the myofibroblast marker a-smooth muscle cell actin. As well, the co-incubation of TWNT4 cells with FA-EV did not lead to an increase in apoptosis.

Conclusion Based on our current observations, we conclud that EV from resting or steatotic HepG2 cells can influence the production of matrix remodeling markers and migration of TWNT4 cells.

1.24 BMP-9 modulates the hepatic responses to LPS

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We have previously shown that Bone Morphogenetic Protein (BMP)-9 is constitutively produced and secreted by hepatic stellate cells (HSC). Upon acute liver damage BMP-9 expression is transiently down-regulated and blocking BMP-9 under conditions of chronic damage ameliorates liver fibrogenesis. Thereby BMP-9 acts pro-fibrogenic in liver but without directly activating isolated HSC in vitro. LPS, an endotoxin derived from the membrane of gram-negative bacteria in the gut, is known to be essential in the pathogenesis of diverse kinds of liver diseases.

Aim of the present project was therefore to investigate how high levels of BMP-9 in the context of LPS signalling might result in enhanced liver damage. For this purpose we stimulated human upcyte® LSEC with LPS and incubated primary human HSC with the conditioned medium of these cells. We found that LPS induced the secretion of factors from LSEC that upregulated BMP-9 expression in HSC. One of these BMP-9 stimulatory factors was found to be IL-6. High BMP-9 in turn induced expression of capillarization markers and latent TGF-β activating proteins in LSEC and enhanced the LPS-mediated induction of pro-inflammatory cytokines in primary human macrophages in vitro as well as in the livers of mice that were injected with BMP-9/LPS.

These data imply that LSEC control the hepatic response to LPS at least in part via regulating the BMP-9 levels in the neighbouring HSC. Our hypothesis is that too much BMP-9 induces fibrosis by promoting LSEC capillarization and TGF-β activity and by provoking too intense inflammatory reactions. Too little BMP-9 on the other hand might disturb liver homeostasis leading to a de-differentiated endothelium and parenchyma. Thereby the direct cross-talk between the non-parenchymal cells, fine-tunes major hepatic responses with BMP-9 being a central homoeostasis-factor.

1.25 Role of NOX1 on hepcidin signaling in the crosstalk between macrophages and hepatocytes

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Background and Aims Liver-secreted hepcidin is the systemic master switch of iron homeostasis and its dysregulation leads to iron accumulation in most of chronic liver diseases. Hepcidin is regulated by iron, inflammation or H2O2, but the role of NOX1 and its products ROS/H2O2 in monocyte-derived macrophages on hepcidin regulation under (patho)physiological conditions is poorly understood. We here investigate the role of NOX1 on regulating hepcidin and cytokines in inflammatory macrophages and subsequent effects on hepatocytes mimicking (patho)physiological conditions (cell ratios, oxygen levels, and inflammation).

Methods THP-1 monocytes were differentiated into macrophages and co-cultured with Huh7 cells at (patho)physiological cell ratios (4:1) and treated with different LPS concentrations (10 ng/ml and 100 ng/ml) under normoxia (21% O2) or hypoxia (1% O2). The exposure of Huh7 cells to macrophage-conditioned medium with LPS was also investigated. Hepcidin, IL-1β, IL-6, C/EBPδ, and SMAD 6 mRNA levels were assessed by qRT-PCR and the expression of NOX1, p-STAT3, STAT3 and p-SMAD 1/5/8 proteins were analyzed by western blot.

Results LPS significantly increased NOX1, p-STAT3, IL-1βand IL-6 levels in THP-1 macrophages, but decreased STAT3 expression in a concentration-dependent manner under 21% and 1% O2. Interestingly, 10 ng/ml LPS increased the expression of hepcidin whereas 100 ng/ml LPS decreased the expression of hepcidin under 21% O2. In contrast, both LPS concentrations decreased the expression of hepcidin 1% O2 in THP-1 macrophages. In addition, LPS decreased SMAD 6, p-SMAD 1/5/8 and CEBPδin THP-1 macrophages under 21% O2. Notably, the treatment of Huh7 cells with LPS had no effect on the expression of IL-6, IL-1β, CEBPδ and hepcidin in Huh7 cells under 21% O2. Using inflammatory macrophage/hepatocyte co-cultures with direct cell-cell interactions under 21% O2 increased IL-6, IL-1β, CEBPδ, and hepcidin expression level in a concentration-dependent manner but did not further increase hepcidin in direct macrophage/hepatocyte co-cultures under 1% O2.

Conclusion Our findings underscore a possible role of NOX1 and subsequent ROS/H2O2 concentrations on hepcidin regulation and induction of cytokine production in inflammatory macrophages involving the STAT3 signaling pathway. In the future, we aim at studying in detail hepcidin signaling by using WT and truncated hepcidin promoter constructs and siRNA-mediated knockdown of TLR4, NOX1, STAT3 or C/EBPδ.

1.26 Mesenchymal stromal cells may promote lipid utilization via increment of mitochondria biogenesis in targeted hepatocytes

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Question In an *in vitro* model of fatty liver, human bone marrow-derived mesenchymal stromal cells (MSC) upregulated genes involved in lipid utilization and, thus reduced the lipid content in the mouse hepatocytes by a mechanism independent of paracrine signaling. Instead, hepatocytes were touched by long filopodium-like, actin- and mitochondria-containing tunneling nanotubes (TNT) derived from MSC. Indeed, the expression of human microtubule motor proteins and related adaptors, namely kinesin family member 5B (KIF5B) and mitochondrial Rho GTPase 1 (MIRO1), were increased in co-culture as compared with MSC alone. We aim to identify the mechanism involved mitigation of hepatocytic lipid load by MSC.

Methods Mono- and co-cultures of primary mouse hepatocytes and human bone marrow-derived MSC were grown in steatosis-inducing methionine-choline-deficient (MCD) medium. To understand the involvement of actin, a major component of TNT, expression of genes involved in actin-dependent TNT transportation and of markers involved in mitochondria biogenesis was examined by RT-PCR using human- and mouse-specific primer pairs.

Results Compared with MSC alone, the co-culture of hepatocytes and MSC did not impact on inducers of actin-based TNT, namely TNFα-induced protein 2 (TNFAIP2) and RAS like proto-oncogene A (RALA), in the MSC. Neither PPARγ coactivator 1α (PPARGC 1A; PGC 1α) nor other markers of mitochondrial biogenesis such as mitochondrial transcription factor A (TFAM) and heme oxygenase-1 (HMOX1) were altered in the MSC. Yet, in the mouse hepatocytes, the expression of PGC 1α, a regulator of mitochondria biogenesis, was enhanced in co-culture as compared with hepatocytes alone.

Conclusion Human MSC may increase the degradation of lipids in mouse hepatocytes by mitochondria transfer from the MSC to the hepatocytes via TNT. Transport may be achieved by microtubule-based, but not actin-based cargo transport. The delivered human mitochondria may induce the biogenesis of mitochondria in the mouse hepatocytes, and thus elevate the expression of genes involved in fatty acid oxidation to elicit lipid utilization. Thus, the results presented here may support the potential of MSC-derived mitochondria transplantation to foster lipid breakdown in fatty livers.

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1.27 Blood reelin levels in the progression of chronic liver disease

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Background Reelin (RELN) is an extracellular matrix protein that was originally discovered as a crucial regulator in tissue architecture in the central nervous system. Recent findings indicate that RELN may play an important role in the organization of other organs as well, especially of the liver. Hepatic RELN expression was shown to be increased during the process of liver fibrosis, moreover there are hints that RELN acts as a tumor suppressor gene in the development of hepatocellular carcinoma (HCC). Against this background, the aim of our study was to explore alterations in blood RELN levels in patients with chronic liver diseases.

Methods We analyzed blood samples of patients with chronic hepatopathy of different etiologies without significant liver fibrosis (n = 25), with significant liver fibrosis (n = 36), with liver cirrhosis (n = 74), with liver cirrhosis and HCC (n = 26) as wells as of healthy controls (n = 15). We also compared blood samples from portal vein, liver vein and peripheral vein of patients with liver cirrhosis (gained during implantation of transjugular intrahepatic portosystemic shunt [TIPS]). Blood RELN concentrations were determined utilizing a human-RELN-specific enzyme-linked immunosorbent assay by Cloud-Clone Corp.®.

Results Blood RELN levels were significantly higher in patients with liver fibrosis or liver cirrhosis than in patients without liver fibrosis or healthy controls (13.2 ± 5.9 ng/ml vs. 18.7 ± 16.9 ng/ml, p = 0.032). Furthermore, patients with liver cirrhosis and HCC displayed significantly higher RELN concentrations compared to patients with liver cirrhosis without HCC (18.7 ± 16.9 ng/ml vs. 27.4 ± 12.8 ng/ml, p < 0.001). This effect was independent of liver function, assessed by the Child-Pugh stadium. We found no significant difference between RELN concentrations in portal vein, liver vein and peripheral veins (14.8 ± 13.1 ng/ml vs. 15.6 ± 14.0 ng/ml vs. 18.8 ± 14.9 ng/ml, p = 0.338).

Conclusions Changes in hepatic RELN expression in chronic liver disease are also reflected in alterations of blood RELN levels, which makes RELN a poten-

tial biomarker in this setting. The pathophysiological mechanisms underlying the changes in blood RELN levels are still under investigation. Further evaluation of RELN as a possible diagnostic marker is a promising task in hepatologic research.

1.28 Growth differentiation factor 11 mitigates liver fibrosis via expansion of liver progenitor cells

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Liver fibrosis and cirrhosis resulting from long-standing liver damage represent a major health care burden worldwide. Members of the transforming growth factor β (TGF-β) family have been shown to play a pivotal role in fibrogenesis of the liver as well as multiple other organs. Growth differentiation factor (GDF) 11, a member of TGF-β family, has been recently investigated for its role in rejuvenation of aging organs. However, the function of GDF11 in liver fibrosis has remained elusive. Here, we investigated the expression and function of GDF11 in chronic liver disease. We show that expression of GDF11 is upregulated in patients with liver fibrosis and in experimentally induced murine liver fibrosis models. Furthermore, we found that therapeutic application of GDF11 mounts a protective response against fibrosis by increasing the number of leucine-rich repeat-containing G-protein coupled receptor 5 (LGR5)+ progenitor cells in the liver. Collectively, our findings uncover a protective role of GDF11 in liver fibrosis and suggest a therapeutic application of GDF11 for the treatment of chronic liver disease.

Poster Visit Session II Clinical Hepatology, Surgery, LTX
Friday, February 14, 2020, 2:40 pm – 3:25 pm, Lecture Hall P1

2.4 Clomethiazole improves alcoholic fatty liver in patients admitted to the hospital for alcohol detoxification therapy

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Chronic alcohol consumption primarily results in alcoholic fatty liver (AFL). Various pathogenetic mechanisms contribute to AFL. The pathophysiology of AFL includes, among others, oxidative stress with the generation of reactive oxygen species (ROS). ROS leads to lipidperoxidation, protein alterations and DNA injury. One important source for ROS is microsomal ethanol metabolism catalyzed by Cytochrome P4502E1 (CYP2E1). Chronic ethanol consumption induces CYP2E1 primarily in the liver, and it has been shown that this induction increases fatty liver by preventing up-regulation of PPARα due to oxidative stress. On the other hand, inhibition of CYP2E1 by clomethiazole (CMZ), a CYP2E1 inhibitor, decreases oxidative stress in cell cultures and improves ALD in animal studies. To study whether CYP2E1 inhibition also improves AFL in humans, we performed a randomized controlled clinical trial in alcohol-dependent patients who were admitted to the hospital for alcohol detoxification therapy (ADT). All patients were non-cirrhotics identified by transient elastography with a liver stiffness of less than 12 kPa and all had serum AST activities of more than twice normal. The patients were randomly assigned for ADT either with CMZ or chlorazepate (CZP) for 7 to 10 days. CMZ almost completely inhibited CYP2E1 already 24 hours after its administration. At admission, controlled attenuation parameter (CAP) was comparable between the two groups (313 ± 11 vs. 310 ± 10 dB/m). At discharge, CAP in the CMZ group was significantly lower as compared to admission (252 ± 11 dB/m; $p < 0.05$), but not in the CZP group (273 ± 8 dB/m; $p = 0.081$). With CMZ, a significant correlation was found between hepatic fat at admission and the difference of fat between admission and discharge ($r = 0.64$; $p < 0.001$). This study proves, for the first time in humans, that CMZ, most likely through the inhibition of CYP2E1, improves AFL more than abstinence alone. Since CMZ can only be given for a short period of time because of its addictive potency, it is mandatory to search for non-toxic CYP2E1 inhibitors to treat AFL.

2.5 Hepatic steatosis in Crohn's disease: associations with anti-TNFα treatment, dysbiosis, and FGF-19.

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Question Non-alcoholic fatty liver disease (NAFLD) is found in up to 33.6% of patients with inflammatory bowel disease (IBD), even in the absence of metabolic risk factors. We and others have shown that NASH in patients with Crohn"s disease (CD) is associated with an increased susceptibility for acute-on-chronic liver failure. Multivariate logistic regression analyses have shown that ongoing anti-Tumor Necrosis Factor-alpha (anti-TNFα) therapy was the only independent protective factor. To date, little is known about the mechanisms leading to hepatic steatosis in IBD nor how anti-TNFα affects steatosis. Therefore, we aimed to analyze and compare non-invasive predictors of liver disease paying special attention to the role of the gut-liver axis and bile-acid metabolism.

Methods We included patients with established CD with and without anti-TNFα treatment and analyzed serum markers of liver injury, transient elastography as well as controlled attenuation parameter (CAP) and MRI proton density fat fraction to assess hepatic steatosis. In addition, we compared gut microbiota and mediators of bile acid (BA) signaling in the absence or presence of treatment with biologicals through analysis of stool and serum from patients and compared it between groups.

Results Patients on treatment with TNFα antibodies expressed lower hepatic steatosis as assessed by CAP and MRI. Serum FGF-19 levels were higher in patients on anti-TNFα treatment and associated with steatosis as well as lower LFT-levels. Group-specific alterations in gut microbiome were found for several bacteria involved in BA metabolism and FGF-19 regulation, including Bacteroides and Firmicutes.

Conclusion Treatment with biologicals protects CD patients from hepatic steatosis. In this cohort, FGF-19 is associated with less steatosis, reduced circulating FGF19 levels and specific alterations in the composition of gut microbiota and BA metabolism.

2.6 Diagnostic algorithm to detect NASH and fibrosis in NAFLD patients with low NAFLD fibrosis score or liver stiffness

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Non-alcoholic steatohepatitis (NASH) and fibrosis play critical roles for the prognosis of patients with non-alcoholic fatty liver disease (NAFLD), making it important to identify patients at higher risk of NASH and fibrosis for optimal disease management. The NAFLD fibrosis score (NFS) and transient elastography (TE) have been suggested as initial diagnostic approach to exclude advanced fibrosis. However, there is increasing evidence that also patients with NASH and early fibrosis are at significant risk of disease progression and complications, emphasizing the need for improved non-invasive risk stratification in NAFLD. Because hepatocyte apoptosis plays an early role in NASH pathogenesis, we evaluated whether the apoptosis biomarker M30, a neoantigen generated by caspase-mediated keratin-18 cleavage, might identify NAFLD patients who are at risk of NASH and fibrosis despite low NFS or TE values. Serum M30 levels were assessed by ELISA in combination with NFS and/or TE in a cohort of 103 biopsy-proven NAFLD patients. The majority of patients with low NFS (cut-off value < -1.455) revealed increased M30 levels (> 200 U/L) and more than 70% of them had NASH, mostly with histological signs of fibrosis. *Vice versa*, most patients with NFS < -1.455 but non-elevated M30 levels showed no NASH. NASH was also detected in most patients with indeterminate NFS (-1.455 to 0.676) but elevated M30 levels, from which 90% showed fibrosis. Similar results were obtained when using TE instead of NFS. In conclusion, the combination of the M30 biomarker with NFS or TE enables a more reliable identification of patients with increased risk of progressed NAFLD and improves patient stratification.

2.7 Isolated bacterial infection without decompensation has no impact on survival of compensated patients

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Background: Patients with cirrhosis can be separated in the compensated and decompensated stage, which has major prognostic relevance. The development of bacterial infections (BI) is common in the natural course of the disease and frequently triggers decompensation. Indeed, BI have been considered as critical events that mark the transition to the decompensated stage. Our aim was to evaluate the impact of BI on the natural history of compensated cirrhosis.

Patients and methods We performed a secondary analysis of a prospectively recruited cohort (n = 858) of consecutive patients with liver cirrhosis, who were screened for the randomized controlled INCA trial (EudraCT 2013 – 001626 – 26) in two academic medical centers between 02/2014 – 05/2019. In- and outpatient medical records were reviewed for present and past decompensation, BI, type 2 diabetes, and inflammatory markers. Only patients with previously compensated disease were included. Applying consensus criteria, BI were defined as infections that required antibiotic therapy and had to be treated in the hospital. The patients were divided into four groups according to their status at baseline: compensated without BI, compensated with BI, 1st decompensation without BI, 1st decompensation with BI. Kaplan-Meier curves were calculated and compared with log rank tests.

Results Overall, 425 patients [median 61 (53 – 69) years old] were included in the analysis, and median follow-up was 372 days (105 – 622). At baseline, 257 patients were compensated (12 [4.9%] with BI), whereas 168 previously compensated patients presented with their 1st decompensation at baseline (42 [25.0%] with BI). MELD score at inclusion did neither differ among compensated patients (no BI 8 [7 – 10], with BI 8 (7 – 11); p = 0.60] nor among patients with first decompensation (no BI 13 [10 – 16], with BI 14 [11 – 21]; p = 0.15). Among patients who remained compensated, BI had no influence on transplant-free survival, but patients with first decompensation plus concomitant BI had significantly (p < 0.001) worse transplant-free survival than those without BI at inclusion.

Conclusions BI without accompanying decompensation has no negative impact on survival of patients with compensated cirrhosis. In contrast, and as previously demonstrated, the first decompensation episode triggered by BI is associated with worst survival. This observation underscores the clinical need for prophylactic strategies to avoid common BI in at-risk patients with cirrhosis.

2.8 Alteration of the gut microbiota in patients with primary sclerosing cholangitis and concomitant dominant strictures

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Question Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease often leading to end-stage liver disease. Its pathogenesis remains largely unknown, although frequent concomitant inflammatory bowel disease (IBD) hints towards common factors underlying gut and bile duct inflammation. Considering the mounting evidence on the involvement of the intestinal microbiota in initiating and determining IBD phenotype, we investigated intestinal microbiota composition in patients with PSC and compared it to patients with IBD.

Methods Stool samples of patients with PSC (n = 49) were collected and compared with samples derived from IBD patients (n = 56) during the time period July 2016 till November 2018 at the University Hospital Heidelberg. At the time of collection all patients were in stable clinical condition without overt signs of infection and without antibiotic treatment for at least three month. Samples were prepared for DNA-isolation and sequenced by ILUMINA-Seq method. In PSC patients subgroups depending on the presence of dominant strictures (DS) or IBD were analyzed. Clinical and laboratory data were collected by chart review.

Results The alpha-diversity is significantly higher in PSC patients due to a more rich microbiome while the dominance is not significantly different. We also observed a significant change in the structure of the microbiome (R^2 = 2%; p-value: 0.016). This change is due to a significant decrease of Firmicutes and increase of Bacteroides in the PSC group. There was no influence of the underlying IBD on the structure. However, the alpha diversity was significantly increased in patients with PSC only compared to PSC patients with concomitant CU (p < 0.01) and CD (p < 0.05) indicating a strong effect of the underlying disease on the microbiome. In subgroup analysis of PSC patients with DS we observed no major impact of the stricture on the alpha and beta-diversity level indicating no changes in the overall structure of the microbiome. There was was a slight change in the structure (R^2 = 0.05, p-value = 0.012) and several species showed a significant difference related to the presence of stricture indicating a mild effect on the biosphere of the microbiome.

Conclusion Gut microbiota composition in PSC patients showes disease specific alterations compared to IBD patients. Differences in the colonic microbiome depending on concomitant IBD or the presence of DS in PSC might be a contributing factor in PSC pathogenesis.

2.9 Evaluation of two functional CD24 polymorphisms in primary sclerosing cholangitis

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Question Primary sclerosing cholangitis (PSC) is a progressive liver disease and characterized by a chronic inflammation, consecutive sclerosis and strictures of the bile ducts. Several genetic risk factors have been described, that might in part contribute to pathogenesis. Functional single nucleotide polymorphisms (SNPs) in the CD24 gene have been associated with the development of several autoimmune and autoinflammatory diseases and might contribute to the susceptibility for inflammatory bowel disease (IBD). We aimed to assess the impact of two common CD24 gene polymorphisms on clinical features and disease progression in patients with PSC.

Methods PSC patients that were treated at our tertiary center between 1987 – 2016 were included into the study. The final study cohort comprises of 359 PSC patients. Two functional CD24 polymorphisms, a C to T coding polymorphism (rs8734) and a TG deletion in the 3'- untranslated region (rs3838646), were genotyped by restriction fragment length polymorphism (RFLP) and melting curve analysis (MCA). Clinical and laboratory parameters were collected by chart review.

Results For the rs8734 genotype, 175 patients (52.2%) were found to be wildtype ("Ala/Ala"), 127 (37.9%) patients were heterozygous ("Ala/Val") and 33 patients (9.9%) were homozygous-mutated ("Val/Val"). Patients carrying homozygous-mutated allels of the rs8734 genotype had less frequent dominant strictures at diagnosis of PSC ($p = 0.04$). For the rs3838646 genotype, 322 patients (89.7%) were found to be wildtype ("TG/TG") and 37 showed the "TG/del" genotype (10.3%). The "TG/del" genotype was associated with a lower risk of inflammatory bowel disease ($p = 0.01$). We found no association of any genotype with the development of a cholangiocarcinoma, gall bladder carcinoma (GBC) or colorectal cancer (CRC). There was no significant difference regarding the combined clinical end point death and liver transplantation or overall survival for both genotypes (rs3838646: $p = 0.8$; rs8734: $p = 0.4$).

Conclusion We found a mild association of the rs8734 CD24 genotype with dominant strictures at first diagnosis of PSC. The rs3838646 CD24 genotype is associated with a lower rate of inflammatory bowel disease in PSC patients. Both SNPs seem to modulate the clinical phenotype without major pathogenetic importance for disease progression in PSC.

2.10 Die Therapie mit Alpha-1-Antitrypsin (AAT) assoziiert bei AAT-Mangel mit besseren Leber-bezogenen Parametern

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Hintergrund Die klassische Form des Alpha-1-Antitrypsin (AAT)-Mangels (Pi*ZZ-Genotyp) stellt aufgrund der Lungen- und Leberbeteiligung eine der häufigsten letal verlaufenden genetischen Erkrankungen dar. Während die intravenöse Augmentation mit AAT die Progression der Pi*ZZ-assoziierten Lungenerkrankung hemmt, stellt die Lebertransplantation bisher die einzige Therapieoption der Pi*ZZ-assoziierten Lebererkrankung dar. Da eine AAT-Gabe *in vitro* zu einer verminderten hepatozytären AAT-Expression führt und da exogenes AAT in experimentellen Mausmodellen hepatoprotektiv wirkt, evaluierten wir den Einfluss der AAT-Augmentation auf den Leber-Phänotyp bei Pi*ZZ-Patienten.

Methodik 481 Pi*ZZ-Probanden ohne hepatische Komorbidität (336 deutsch, 62 österreichisch, 40 portugiesisch, 25 dänisch und 18 spanisch), davon 206 ohne und 275 mit AAT-Augmentation, erhielten eine systematische klinische und laborchemische Untersuchung. Die Lebersteifigkeit (LSM) wurde mittels transienter Elastografie bestimmt. Die Daten wurden für Alter, Geschlecht, BMI und Diabetes mellitus adjustiert.

Ergebnis Pi*ZZ-Probanden, die eine AAT-Augmentation erhielten, waren älter und hatten eine ausgeprägtere pulmonale Symptomatik (höherer CAT (COPD Assessment Test) und häufiger Heimsauerstofftherapie) als Nicht-augmentierte. Dahingegen zeigten Augmentierte niedrigere GGT- und AST-Werte (88 vs. 100 U/L, $P = 0.008$ und 77 vs. 71 U/L, $P = 0.004$). Außerdem wiesen sieeine geringere Lebersteifigkeit (6.1 vs. 6.7 kPa, $P = 0.015$) sowie verminderte APRI- und FIB-4-Werte (0.31 vs. 0.34 units, $P = 0.001$ und 1.28 vs. 1.38 units, $P < 0.0001$) auf. Nur 10,6% der Augmentierten hatten LSM ≥ 10 kPa, hinweisend auf eine fortgeschrittene Leberfibrose, im Vergleich zu 15,8% der Nicht-augmentierten ($P = 0.005$). Zudem zeigte sich, dass eine längere Augmentation mit niedrigeren LSM assoziiert war (> 5 Jahre: 5.7 kPa, > 10 Jahre: 5.4 kPa, jeweils $P < 0.02$ vs. Nicht-augmentierte (6,7 kPa)). Eine multivariable Analyse bestätigte die Assoziation zwischen der AAT-Augmentation und der Verbesserung dieser Leber-bezogenen Parameter.

Schlussfolgerung In einem Querschnitt einer großen Pi*ZZ-Patientenkohorte zeigte sich, dass die AAT-Augmentationstherapie positive Auswirkungen auf die Pi*ZZ-assoziierten Leberphänotypen haben könnte. Weitere, prospektive Studien sind nötig, um diese Observation zu bestätigen und die zugrundeliegenden Mechanismen aufzudecken.

2.11 Non-malignant portal vein thrombosis in liver cirrhosis – predictors of risk and the use of anticoagulation

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Introduction The occurrence of a portal vein thrombosis (PVT) in patients with liver cirrhosis is a controversial discussed topic. The effect on the natural course of the underlying liver disease and the possible role as a negative predictor has not yet been fully clarified.

Aim of study This study aimed to determine possible predictors for higher mortality in patients with liver cirrhosis and portal vein thrombosis and to investigate the impact of established therapies.

Methods Approximately 30.000 cross-sectional imaging CT- and MRI-scans in a single transplant center (University hospital Mainz) were retrospectively screened for portal vein thrombosis with underlying liver cirrhosis. Cases with any malignancy were excluded from further analysis. Mortality was correlated with clinical and laboratory findings at the time of PVT diagnosis and throughout the end of observation period.

Results 55 patients with liver cirrhosis and PVT were included into analysis. The median follow-up time was 129 weeks and median age at diagnosis of PVT 57.6 years. Median MELD at PVT diagnosis was 13. During follow-up 35 patients died (63.6%) after 68 weeks in median. Using univariable cox proportional-hazard regression models, MELD (HR 1.07, p = 0.009), lower serum albumin levels (HR 0.885, p = 0.001), ascites (HR 2.22, p = 0.031), sponta-

neous bacterial peritonitis (SBP) during follow-up (HR 2.25, p = 0.020), overt hepatic encephalopathy at diagnosis of PVT or during follow-up (HR 3.11, p = 0.005), and hepatorenal syndrome at diagnosis of PVT or during follow-up (HR 3.43, p = 0.001) were identified as predictors for higher mortality. Therapeutic anticoagulation tended to improve prognosis (HR 0.5, p = 0.055). In a multivariable cox-regression model, SBP during follow-up (HR 7.75, p = 0.0001) and lower serum albumin levels (HR 0.78, p = 0.0001) were shown to be independently associated with a lethal course. Additionally, after adjusting for the aforementioned variables, therapeutic anticoagulation was an independent predictor for better prognosis (HR 0.128, p = 0.0001).

Conclusion Spontaneous bacterial peritonitis and lower albumin levels are predictors for high mortality in patients with liver cirrhosis and PVT. Additionally, anticoagulation was an independent predictor for better prognosis in these patients. Consequently, future studies should evaluate the use of anticoagulation in every patient with liver cirrhosis and PVT.

2.12 Relapse rates in patients with autoimmune hepatitis after withdrawal of medication.

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Autoimmune hepatitis is a chronic inflammatory disorder of the liver. The current work aimed to evaluate the time periods for relapse after withdrawal of immunosuppressive medication due to side effects or patient"s wish, in a real life setting. Also, predictive factors for outcome were evaluated. A total of 75 patients was investigated from January 2007 until July 2017 at Helios Universitätsklinikum Wuppertal. The clinical development after discontinuation or discontinuation of maintenance therapy was evaluated.

Overall, time period for evaluation of relapse rates after withdrawal ranged at 18 – 114 months. From the 75 treated patients who discontinued medication, 51 patients (68%) had a relapse after an average of 8.6 months, 24 patients (32%), however, maintained a remission without further medication. Out of 14 patients who received a monotherapy for conservation with azathioprine, 12 patients (85.71%) had a relapse after an average of 7,1 months. Only three out of 9 patients (33,33%) in the prednisolone mono-group presented a recurrence after an average of 9 months. Of the 23 patients treated with budesonide monotherapy patients, a total of 14 (60,9%) had relapse after an average of 11.9 months. 12 (75%) of 16 patients which maintenance therapy with azathioprine and prednisolone had a relapse after an average 4.6 months. From the group of patients with a combination therapy of azathioprine and budesonide relapsed 4/6 (66,67%) patients after ~13.5 months. Of the two patients who received cyclosporine, both had a relapse after 11,5 months following discontinuation of therapy. Thus, the risk of relapse was 0.2 times lower for those treated with budesonide compared to the group treated with azathioprin (p = 0,01). The combination of azathioprin and budesonide seems to provide a 0,204-times lower Risk to agonize/gain a relapse as when using only azathioprin (p-value = 0,030).

Withdrawal of immunosuppressive medication is associated with approximately 60% risk of relapse in AIH. The highest risk for relapse was found in the group treated with azathioprine monotherapy, while prednisolone treatment over more than 3 years led to a lower relapse rate. Therefore, the combination-therapy of azathioprine/prednisolon or azathioprine/budesonide as well as monotherapy with prednisolon compared to azathioprine monotherapy was shown to be superior with regard to the appearance of relapse.

2.13 Extrakorporale Leberunterstützung mit ADVOS in Patienten mit ACLV und HRS – Erste Ergebnisse einer Single Center Studie

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Das Akut-auf-chronische Leberversagen (ACLV) ist ein komplexes Krankheitsbild mit einer hohen Mortalität und hohen Kosten für das Gesundheitssystem. Die Hepatische Enzephalopathie (HE) und das Hepatorenale Syndrom (HRS) sind häufige Komplikationen und wesentliche Mortalitätsprädiktoren des ACLV. Die therapeutischen Optionen für Patienten, bei denen eine Lebertransplantation nicht möglich ist, sind limitiert. Der Einsatz von extrakorporalen Leberunterstützungsverfahren kann möglicherweise den Zeitraum bis zur Stabilisierung der Organfunktionen oder einer Transplantation überbrücken. Bei ADVanced Organ Support (ADVOS) werden die albumingebundenen Stoffe im Gegensatz zu den in der Vergangenheit eingesetzten Verfahren durch Änderung des pH-Milieus abgelöst und mit den wasserlöslichen Toxinen eliminiert. Die Regeneration des eingesetzten Albumins schont Ressourcen und spart Kosten. ADVOS wird meist als kontinuierliches Verfahren auf Intensivstationen eingesetzt. In der vorliegenden Studie wurden die ersten 6 ACLV-Patienten mit HRS auf Effektivität einer intermittierenden ADVOS-Therapie untersucht. Ziel war die Evaluation der Veränderung von Bilirubin, Kreatinin und Harnstoff sowie der Einfluss auf die HE. Die Patienten wurden mit der 8 h-ADVOS-Therapie über einen Zeitraum von 14 Tagen behandelt. Nach zunächst täglicher Therapie wurde nach dem 5. Tag auf ein übertägiges Regime umgestellt. Die HE wurde mittels psychometrischem HE-Score (PHES) und kritischer Flimmerfrequenz (CFF) getestet. Das mittlere Alter der Patienten (alle männlich) lag bei 57 Jahren (IQA 5,25), der mittlere MELD-Score betrug 34 (IQA 9). Die Patienten erhielten im Schnitt 8,5 Therapien (IQA 3,5) mit einer mittleren Behandlungszeit von 3878 min (IQA 1497). Bilirubin wurde um 12% reduziert (24,7 ± 11,5 vs. 22,4 ± 11,8 mg/dl, p > 0,05), Harnstoff sank um 28% (61,1 ± 17,5 vs. 39,3 ± 12,1 mg/dl, p < 0,05), Kreatinin verzeichnete einen Anstieg (3,9 ± 2,5 vs. 4,1 ± 1,0 mg/dl, p > 0,05). Der PHES-Score und die CFF blieben stabil. Das 28 d-Überleben betrug 50%, mit der Sepsis als führende Todesursache. In dieser Pilotstudie von 6 ACLV-Patienten mit HRS konnte die intermittierende ADVOS-Therapie die Bilirubin- und Harnstofflevel effektiv reduzieren. Die HE besserte sich klinisch, die Testungen zeigten jedoch nur einen marginalen Effekt. Die intermittierende ADVOS-Therapie stellt ein probates Mittel zur Behandlung von ACLV-Patienten dar. Weitere Studien sind erforderlich, um die Effekte näher zu untersuchen.

2.14 Antibiotische Therapie von Leberabszessen: Enterokokken sind eine häufige Ursache für ein Therapieversagen

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Hintergrund: Die Therapie eines Leberabszesses beruht neben der Drainage und ggf. chirurgischen Intervention als einem weiteren Hauptpfeiler auf einer empirischen Antibiotikatherapie, die in der Regel als kalkulierte Therapie erfolgt. Voraussetzung für eine suffiziente anti-infektive Therapie ist dabei die Kenntnis über das lokale Erreger- und Resistenzspektrum sowie über Risikofaktoren für das Vorhandensein von Resistenzen.

Methoden: Es wurden alle Patienten, die zwischen 2014 und 2019 in unserer Klinik behandelt wurden, eingeschlossen. Die entweder mittels Punktion des

Abszesses gewonnenen Kulturen wurden retrospektiv auf Erregerverteilung, -häufigkeit und vorliegende Resistenzen analysiert. Daten zur Antibiotikatherapie, klinische, laborchemische und anamnestische Daten wurden zur Identifikation von Risikofaktoren für Resistenzen erhoben.

Ergebnisse: Bei 37 (65%) Patienten gelang ein Erregernachweis. Bei 14 Patienten konnte E. coli (37,9%), bei 13 Enterococcus spp. (35,1%), davon 3 VRE, bei 7 Streptococcus spp. (19%) und bei weiteren 7 Klebsiella spp. (18,9%) nachgewiesen werden. In 2 Fällen wurde eine Amöbiasis festgestellt. Insgesamt war die kalkulierte Antibiotikatherapie in 21 (56,8%) der 37 Patienten mit positivem Erregernachweis nicht vollständig effektiv und bei 24 (64,9%) dieser Patienten wurde die antibiotische Therapie nach Kenntnis des Antibiogramms umgestellt. Bei E. coli-Nachweis war die kalkulierte Antibiotikatherapie bei 3 Stämmen nicht effektiv (17,7%), während bei Enterococcus faecium bei 8 (72,7%) und bei Enterococcus faecalis bei 4 (80%) der Stämme eine ineffektive empirische Antibiotikatherapie erfolgte. Patienten mit Enterokokkennachweis zeigten einen längeren Krankenhausaufenthalt (p = 0,02), eine längere Antibiotikatherapiedauer (p = 0,013) und ein erhöhtes Risiko für eine chirurgische Intervention (p = 0,004). Risikofaktoren für eine Enterokokkeninfektion waren eine hepatobiliäre (p = 0,007) oder pankreatische (p = 0,034) Vorerkrankung, sowie eine Gallengangsintervention in den letzten 90 Tagen (p = 0,019).

Schlussfolgerung: Infolge häufigen Auftretens von Enterokokken bei pyogenen Leberabszessen und damit verbundenen Komplikationen sollte bei Vorliegen von Risikofaktoren die empirische Antibiotikatherapie mit Piperacillin/Tazobactam um eine Enterokokken-wirksame Substanz wie Vancomycin oder Linezolid erweitert werden.

2.15 RIPK1 and MLKL in hepatocytes do not mediate Concanavalin A (ConA)-mediated liver toxicity

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Apoptosis and necrosis are distinct forms of hepatocyte death and prominent features of liver diseases. In contrast to apoptosis, which seems to be the dominant form of hepatocyte death in most models of hepatitis, ConA-injection is associated with immune cell-mediated inflammation and hepatocyte necrosis. So far it is still unclear how ConA induces liver necrosis and which molecules are involved in this process. Previous studies indicate that necroptosis, a regulated form of necrosis that involves activation and complex formation of RIPK1, RIPK3 and MLKL, occurs after ConA-application. The studies indicate that especially RIPK1 and MLKL are critical mediators of ConA-mediated liver damage, whereas RIPK3 is not involved in this process. However, the interpretation of these results is partly difficult due to poor strain-matching of control and knock-out (KO) mice, the investigation of just a single time point after Injection or potential off-target effects of inhibitors like the RIPK1 kinase inhibitor necrostatin-1 s. Therefore it is still unclear if necroptosis is involved in ConA-mediated liver toxicity.

To evaluate the role of RIPK1 and MLKL in this context in more detail we used mice with a conditional ablation of RIPK1 or MLKL in liver parenchymal cells ($RIPK1^{LPC\text{-}KO}$ and $MLKL^{LPC\text{-}KO}$) and investigated the amount of necrotic damage after ConA-injection compared with the respective Wildtype (WT) litter mates.

Intravenous ConA-treatment for 7 h, 24 h or 48 h did not reveal any differences in liver enzymes and the degree of necrotic liver damage in $RIPK1^{LPC\text{-}KO}$ and $MLKL^{LPC\text{-}KO}$ mice arguing against a hepatocyte-specific function of RIPK1 and MLKL in ConA toxicity. These obvious differences to previous studies emphasize the importance of the use of specific conditional KO mice and indicate that the interpretation of results generated with constitutive KO mice or by the use of systemic inhibitors must be interpreted with caution. Considering all this data it cannot be excluded that other intra- and extra-hepatic cell compartments than hepatocytes are involved in ConA-mediated liver damage. To stimulate the discussion on this topic it would be therefore helpful to analyze the effect of ConA in mice lacking RIPK1 or MLKL in other cell compartments. Taken together, our findings indicate that hepatocytic RIPK1 and MLKL do not modulate acute liver toxicity of ConA in mice. Furthermore they provide evidence for a lack of function of necroptosis in this process.

2.16 Systemic inflammation is associated with hyperdynamic circulation and predicts acute-on-chronic liver failure

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Background & Aims Acute-on-chronic liver failure (ACLF) is characterized by high short-term mortality and systemic inflammation (SI). Recently, different cardiodynamic states were shown as independent predictors of outcomes in cirrhosis. The relationship between cardiodynamic states, SI and portal hypertension and their impact on ACLF development have not been fully studied. The aim of the present study was therefore to evaluate the interplay of cardiodynamic state, fatal ACLF development and SI in liver cirrhosis.

Approach & Results At inclusion, haemodynamic measures including the cardiac index (CI) and hepatic venous pressure gradient (HVPG) of 208 cirrhotic patients were measured. Patients were followed prospectively for fatal ACLF (primary endpoint). SI was assessed measuring the levels of proinflammatory interleukins (ILs) 6 and 8 and IL-33 receptor – soluble ST2.

Patients were divided according to CI (< 3.2; 3.2 – 4.2; > 4.2 L/min/m2) in hypo- (n = 84), normo- (n = 69) and hyperdynamic group (n = 55). After a median follow up of 3 years, the highest risk of fatal ACLF was seen in hyperdynamic (35%) and hypodynamic patients (25%) compared to normodynamic (14%) (p = 0.011). Hyperdynamic state showed highest rate of SI. The detectable level of IL-6 was an independent predictor of fatal ACLF development.

Conclusions Cirrhotic patients with hyperdynamic and hypodynamic circulation, have a higher risk of fatal ACLF. Hyperdynamic state is strongly associated with elevated markers of systemic inflammation, which independently predict fatal ACLF development.

2.17 Predictive parameters and effectiveness of TIPS reduction in treatment of hepatic encephalopathy after TIPS

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Question Aim of this retrospective study was to analyze the effectiveness of technical and clinical success of transjugular intrahepatic portosystemic shunt (TIPS) reduction for the treatment of hepatic encephalopathy (HE) after TIPS

procedure. We further aimed to find predictive parameters for the development of HE in patients receiving TIPS.

Methods We analyzed data of 93 patients who received a TIPS at University Medical Center of Münster of which 45 patients developed HE after TIPS and 48 patients did not. 44% of patients who had developed HE after TIPS received TIPS reduction for the treatment of HE. We classified patients as responders to TIPS reduction if they achieved at least one stage improvement of HE according to West Haven Criteria. Patients who did not achieve at least one stage improvement were classified as non-responders.

Results Technical success rate of TIPS reduction was 100%. Mean increase of portosystemic pressure gradient (PPG) was 5 mm Hg [7 mm Hg (1 – 17) to 12 mm Hg (6 – 21)]. Treatment of HE via TIPS reduction was successful in 55% of patients (responders). Recurrence of refractory ascites or variceal-bleeding requiring treatment was not observed. Six-month mortality rate was 33.3% in non-responders and only 9.1% in responders to TIPS reduction. High stage of HE was identified as a positive predictive variable for treatment success of HE via TIPS reduction. Increased serum creatinine levels after TIPS procedure, high INR as well as older patient age were identified as independent variables for development of HE after TIPS procedure.

Conclusion TIPS reduction is a safe and potent procedure for treatment of HE after TIPS. Patients with higher stage of HE can profit from TIPS reduction, independent from PPG. Serum creatinine level, high INR and patient age should further be considered as predictive variables for development of HE after TIPS procedure and deserve attention in the selection of patients eligible for TIPS.

2.18 Endokrine Störungen bei Leberzirrhose: häufig, aber wenig beachtet

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Hintergrund Bei Patienten mit Leberzirrhose sind häufig klinisch Malnutrition und ein kataboler Status festzustellen. Gleichzeitig werden wegen der Leberdysfunktion sowohl die Synthese als auch der Abbau von Hormonen beeinträchtigt, was deren Konzentrationen in komplexer Weise beeinflusst. Die unzureichende Versorgung mit Vitaminen und die Hormonstörungen verursachen häufig nur unspezifische Symptome, die von denen der Zirrhose schwer zu differenzieren sind. Daher untersuchten wir jetzt bei Patienten mit Leberzirrhose die Hypothese, dass es sich bei diesen Störungen um unterdiagnostizierte und nicht ausreichend behandelte Veränderungen handelt.

Patienten und Methodik In einer prospektiven Beobachtungsstudie bei Patienten mit neu diagnostizierter Leberzirrhose werden endokrinologische Parameter (Cortisol, ACTH, IGF-1, HbA1c) und die Konzentrationen des Vitamin D-Hormons sowie der anderen fettlöslichen Vitamine im Nüchternzustand zwischen 08:00 und 10:00 Uhr erfasst.

Ergebnisse Insgesamt wurden aktuell 23 Patienten mit Leberzirrhose unterschiedlicher Ätiologie im Child-Pugh-Stadium A bis C rekrutiert. Jeweils fünf Patienten (22%) hatten HbA1c-Werte, die diagnostisch für Prädiabetes (5,7 – 6,4%) oder Diabetes waren (> 6,5%). Die Messwerte von Cortisol und ACTH lagen bei allen Patienten im Referenzbereich (Cortisol-Mittelwert 11,9 µg/dl). Dagegen wiesen 57% der Patienten (13/23) einen IGF-1-Mangel auf (Mittelwert 49,4 ng/ml, Referenzbereich 45 – 210 ng/ml). Mit einem Mittelwert von nur 11,4 ± 4,6 ng/ml wiesen alle Patienten eine unzureichende Versorgung mit Vitamin D (Referenzbereich 30 – 100 ng/ml). 83% der Patienten (19/23) hatten auch eine unzureichende Versorgung mit Vitamin A (Mittelwert 19,6 ± 9,1 µg/dl, Referenzbereich 30 – 70 µg/dl).

Diskussion Diese Pilotstudie weist auf eine hohe Prävalenz eines IGF-1-Mangels in Kombination mit einer unzureichenden Versorgung mit Vitamin A und D sowie gestörter Glukosetoleranz oder Diabetes mellitus bei Patienten mit Leberzirrhose, die sich in einem universitären Zentrum vorstellen, hin. Weitere Studien sind erforderlich, um zu klären, ob der Vitaminmangel und die Hormonstörungen eine Folge der Zirrhose sind und welche funktionellen Wechselwirkungen die endokrinen Störungen haben.

2.19 Fibroseprogredienz bei Patienten mit Budd-Chiari Syndrom

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Das Budd-Chiari-Syndrom (BCS) ist die Folge des Verschlusses der Lebervenen. Die Abflussstörung führt im akuten Stadium zur portalen Hypertension mit starker Aszitesbildung, die mit einer TIPS-Anlage behandelt werden kann. Während des chronischen Verlaufs, kann die anhaltende Perfusionsstörung der Leber die Entstehung von zentrilobulären Nekrosezonen, Regeneratknoten und Fibrose verursachen. Über die Geschwindigkeit der Progression der Fibrose bis hin zur Transplantationsbedürftigkeit ist nicht viel bekannt. In der vorliegenden Studie wurde bei 19 Patienten, mit BCS die Fibroseprogression im Langzeitverlauf durch eine Fibroscanuntersuchung bestimmt.

Methoden Sechs (32%) der 19 Patienten waren männlichen Geschlechts, das Lebensalter betrug 38 ± 13 Jahre (median 38,7; 16 – 61 Jahre). Da die Patienten vor dem akuten BCS keine Lebererkrankung aufwiesen, wurde als Basiswert ein Normwert von 5 kPa angenommen. 8 Patienten erhielten einen transjugulären Shunt (TIPS) als akute Maßnahme (innerhalb von 4 Wochen nach Symptombeginn), 9 Patienten als verzögerte Maßnahme (72 bis 2265 Tage nach Symptombeginn) und 2 Patienten erhielten nur eine Antiokoagulation. Im Verlauf von 11 ± 7 Jahren (median 10,5; 2 – 21 Jahre) wurden 1 – 5 Fibroscanuntersuchungen pro Patient durch denselben Untersucher vorgenommen und der Fibroseprogressionsindex, (FPI) wie folgt bestimmt: (gemessene kPa-5):Jahre seit Diagnosestellung.

Ergebnisse Insgesamt wurden 52 Fibroscanmessungen bei 19 Patienten vorgenommen. 7 Patienten hatten einen mittleren FPI von < 1 kPa/Jahr (0,19 – 0,91), bei 8 Patienten betrug der FPI < 4 kPa/Jahr (1,17 – 3,58) und 4 Patienten hatten einen hohen FPI mit > 6 kPa/Jahr (6,23 – 11,6). 11 der 19 Patienten erreichten kPa-Werte von > 15 (15,9 – 49,6) und hatten somit vermutlich eine Zirrhose. Da 17 der 19 Patienten eine TIPS-Implantation erhalten hatten, waren Varizenblutungen oder Aszites nur bei Shuntinsuffizienz aufgetreten. Kein Patient hat Zeichen einer signifikanten Leberfunktionsstörung oder hepatische Enzephalopathie entwickelt, kein Patient ist verstorben oder transplantiert worden.

Diskussion Der Fibroseprogressionsindex (FPI) nach BCS lässt den Schluss zu, dass jeweils etwa 40% der Patienten einen sehr milden oder moderaten Verlauf haben und keine oder sehr spät auftretende Zirrhose erleben werden. Etwa 20% der Patienten zeigten eine rasche Progression zur Zirrhose. Diese kleine Gruppe wird je nach Alter bei Diagnosestellung eventuell eine Lebertransplantation benötigen.

2.20 Outcome prediction of covert hepatic encephalopathy in liver cirrhosis: comparison of four testing strategies

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Question Despite the negative impact of covert hepatic encephalopathy (CHE) on the outcome of patients with liver cirrhosis, data regarding the ability of different testing strategies to predict overt hepatic encephalopathy (OHE) development and mortality are limited. This study aimed to compare the ability of Psychometric Hepatic Encephalopathy Score (PHES), critical flicker frequency (CFF), simplified animal naming test (S-ANT1) and Clinical CHE (CCHE) score to predict OHE development and mortality.

Methods 367 patients with liver cirrhosis were screened for this prospective study between March 2017 and December 2018 at the University Medical Center in Mainz and the Diakonie Klinikum Siegen. Patients were excluded if they fulfilled one of the following criteria: previous episode of OHE during the last three months, the presence of malignancies, chronic alcohol consumption or neurological comorbidities. All patients were tested with the different testing strategies and prospectively followed regarding clinically relevant outcomes (OHE or death/liver transplantation).

Results A total of 252 patients with liver cirrhosis were enrolled in Mainz and Siegen. Follow-up data were available for 224 patients with a median follow-up time of 364 days (IQR 202; 508). During follow-up, 39 patients (17.0%) developed an episode of OHE and 45 patients died or received a liver transplantation. The majority of the patients were male (56.7%) with a median MELD score of 10 (7; 14). Prevalence of pathological results varied among the testing strategies: PHES 33.9%, CFF 17.9%, S-ANT1 41.5%, and CCHE score 33.9%. All testing strategies were independent predictors of OHE development after adjusting for MELD score and history of OHE. The predictive performances of PHES (AUROC, 0.742) and CCHE (AUROC, 0.785) regarding OHE development during the next 180 days were significantly better than of CFF and S-ANT1. In multivariable analysis, pathological results in PHES, S-ANT1 and CCHE were independently associated with higher mortality. CFF did not correlate with mortality in the whole cohort. In the subgroup of patients with a MELD score < 15, pathological results in PHES, CFF or CCHE were independent predictors of higher mortality.

Conclusion PHES and CCHE score predict OHE development and mortality in patients with liver cirrhosis. In particular, in patients with low MELD score both testing strategies could help to identify patients who may benefit from liver transplantation.

2.21 Whole exome sequencing analysis revealed an inherited AKR1D1 defect in twins with infantile intrahepatic cholestasis

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Neonatal cholestasis comprises a variety of disorders, including inherited defects in bile acid (BA) synthesis, conjugation or transport. A family presented with twins at the age of 2 months with elevated transaminases, hyperbilirubinemia, and infantile cholestasis. The mother had experienced an episode of cholestasis after ingestion of ecstasy. Whole Exome Sequencing (WES) was performed from both parents and the twins.

For data analysis, an autosomal recessive disorder was assumed. As the parents were not consanguine, compound heterozygosity seemed plausible. WES revealed 40,000 genetic variants for each twin. Eliminating all synonymous variants, over 10,000 variants per patient remained. For further analysis, heterozygous variants were selected (6,300 variants remaining) and filtered for heterozygosity in the parents (4,400 variant remaining). Next, genes with more than one variant were selected, leaving us with 2,300 variants, which were filtered for minor allele frequencies of < 1% and potentially pathogenicity by *in silico* prediction using PolyPhen2 (50 variants remaining). Of these, both children showed the same two variants within the AKR1D1 gene, whereas each parent had one of these variants. DNA analysis for the healthy sister revealed homozygosity for the wildtype allele at both positions.

AKR1D1 variants are associated with delta(4)-oxosteroid-5beta-reductase deficiency, resulting in a lack of primary BA and an accumulation of atypical 3-oxo-delta-4 and allo-bile acids, which can be measured in urine. These abnormalities were present in these patients and cholic acid supplementation was initiated and led to improvement of liver function in both children.

This case of a family with an inherited bile acid synthesis deficiency illustrates the importance of a combined interdisciplinary clinical, genetic, and biochemical analysis for diagnostics of infantile cholestasis.

2.22 Vitamin A deficiency is associated with disease severity in patients with liver cirrhosis

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Background Malnutrition and subsequent development of cachexia is frequently observed in patients with liver cirrhosis. Subsequent induction of oxidative stress within the liver is associated with increased morbidity and mortality. Malnutrition-induced deficiency of the anti-oxidative vitamin A may, thus, be a modifiable factor and contribute to the progression of chronic liver diseases.

Methods From March 2018 to May 2019, vitamin A status of 161 patients with liver cirrhosis were prospectively assessed at our outpatient and inpatients liver unit at the University Medical Center Mainz. Clinical and laboratory parameters were recorded and associated with vitamin A levels.

Results Vitamin A deficiency was found in 67% of cirrhotic patients. Vitamin A deficiency showed an inverse correlation with labMELD values (R = -0.6, p value = 0.001) and direct correlation with parameters of the liver function (albumin, R = 0.8, p value =< 0.001; bilirubin R = -0,04, p value =< 0.001; INR, R = -0,6, p value =< 0.0001). Likewise, patients with a history of hepatic encephalopathy (p value = 0.05) or signs of decompensation (p value = 0.03) showed a significant reduction in vitamin A deficiency. CRP as a marker for inflammation and infection also correlates significantly with serum vitamin A levels (R = -0.37, p value =< 0.0001).

Conclusion Majority of cirrhotic patients suffer from vitamin A deficiency. Herein, the liver synthesis correlates significantly with the extent of vitamin A deficiency. Vitamin A deficiency is particularly pronounced in patients with ascites and hepatic encephalopathy.

2.23 Extending the reach of the ALBI score for primary hepatic malignancies: Does ALBI work for cholangiocarcinoma?

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Purpose While several prognostic scores for patients with resectable intrahepatic cholangiocarcinoma (ICC) exist, patients with non-resectable ICC are less well studied. Recently, the albumin-bilirubin (ALBI) score was shown to be a prognostic marker for patients with resectable ICC, too. Aim of this study was evaluate the prognostic impact of the ALBI score on overall survival (OS) for patients with non-resectable ICC.

Methods and materials Between 1997 – 2018, 417 patients with ICC were referred to our tertiary care centre and were retrospectively identified out of a dedicated clinical database, of whom 158 patients were non-resectable and could be included in this study. The ALBI score [(log10 bilirubin × 0.66) + (albumin ×-0.085), with bilirubin in µmol/L and albumin in g/L] was calculated and compared to the current UICC staging system regarding its predictive ability for OS. Moreover, the ALBI score was compared to established risk fators and imaging parameters using multivariate Cox hazard regression.

Results Median OS was 13.8 months, 6.9 months, and 1.8 months for patients with ALBI grade 1, 2, and 3 respectively ($p < 0.001$). Concordance index (C-index) calculation yielded 0.65 compared to a c-index of 0.62 for the UICC staging system. In multivariate analysis including tumour number and spread, laboratory markers, and subsequent treatment, the ALBI score remained a significant predictive factor for survival (hazard ratio 1.5 for grade 2 and 3.0 for grade 3, $p = 0.08$ and $p = 0.001$, respectively).

Conclusion The ALBI score was highly predictive for OS in patients with non-resectable ICC, despite having been developed for patients with hepatocellular carcinoma. Due to its simplicity, the ALBI score can easily be applied in clinical routine.

2.24 Low muscle mass and large portosystemic shunt in "one-stop shop" CT exponentially increases risk for HE and mortality

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Background Sarcopenia and spontaneous portosystemic shunts are common complications of liver cirrhosis and both are associated with higher rates of hepatic encephalopathy (HE) development in these patients. This study aimed to evaluate the simultaneous impact of skeletal muscle mass and spontaneous portosystemic shunting, measured from routine diagnostic computed tomography (CT), on outcome in patients with liver cirrhosis.

Methods Consecutive patients with liver cirrhosis presenting to our center were retrospectively evaluated. Skeletal muscle mass (including fat-free muscle index (FFMI) as surrogate for sarcopenia) and total cross-sectional spontaneous portosystemic shunt area (TSA) were quantified from available CT scans. Primary endpoint was development of HE, secondary endpoint was all-cause mortality within 1-year follow-up.

Results Among 209 patients included, patients with low (L-)FFMI and large (L-)TSA showed higher rates of HE development. In multivariable analysis, L-FFMI and L-TSA were independent predictors of HE development (L-FFMI HR = 2.68; L-TSA HR = 2.48) and 1-year mortality (L-FFMI, HR = 7.68; L-TSA, HR = 3.05). Simultaneous presence of L-FFMI and L-TSA exponentially increased the risk of HE development (HR = 12.7) and 1-year mortality (HR = 13.66).

Conclusion This study is the first to indicate a potential synergy between low skeletal muscle mass and large portosystemic shunting to predict exponentially increased risk of HE development and mortality in liver cirrhosis. Simultaneous opportunistic screening for sarcopenia and portosystemic shunt area from routine diagnostic CT may help to improve identification of high-risk patients.

2.25 Slow progressive very late-onset LAL-D: the importance of liver biopsy

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Question/Clinical Case A 68-year old woman presented to our center for further evaluation of elevated liver function tests. She reported a 20 years history of elevated liver enzymes. Histology results similarly demonstrated microvesicular fatty liver with minimal fibrosis. Re-examination of a biopsy taken in 2008 did already show foamy macrophages which had been overlooked at that time. In line with the patient's metabolic profile, her case was treated as NASH-related although histological criteria were not fulfilled completely.

Methods After referral to our center, we performed enzymatic dry blood spot (DBS) test which showed reduced Liposomal Acid Lipase (LAL) activity. Another liver biopsy was taken which now showed fibrosis stage III, a mixed pattern of steatosis as well as discernible macrophages transformed into foamy cells. In addition, we had the unique opportunity to perform electron microscope (EM) examination, which is a rare possibility for this disease.

Results EM pictures revealed pathognomonic membrane-bound lipid drops within the lysosome of hepatocytes clearly proving the diagnosis of LAL-D. Subsequently, treatment with Sebelipase alpha was initiated.

Conclusions Usually, the median age of first reported manifestation of LAL-D is around 5 years and 83% of cases manifested by an age of 12. However, disproportionately younger age of LAL-D patients compared with the general population suggests that many LAL-D patients may be missed; possibly even die prior to diagnosis. Our patient had a slowly progressive form although no enzyme activity was detected. Indeed, it took her more than 20 years to progress from stage I to III fibrosis. This slow progression is actually a hallmark in NAFLD.

2.26 Symptom burden and treatment response in patients with primary biliary cholangitis (PBC)

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Background Primary biliary cholangitis (PBC) is a chronic, cholestatic liver disease that can lead to end-stage liver disease, provoking not alone physical symptoms.

Aim: This cross-sectional study evaluated treatment response and symptom burden in PBC patients with early and advanced disease in Germany.

Methods A total of 151 PBC patients were prospectively enrolled at the outpatient liver clinic of the University Medical Center in Mainz starting in June 2016. By chart review, historic response rates 12 months after initiation of UDCA treatment were determined using published binary models. Symptom burden was assessed using the PBC-40 questionnaire.

Results The prevalence of early disease with normal bilirubin, INR and albumin was 69%. Treatment response ranged between rates at 12 months ranging from 45% according to Barcelona up to 88% according to Paris-I-criteria. Importantly, this rate was maintained over a median time of 9 years. The symptom burden was high across all scored categories with the domains fatigue and emotional (2.4 ± 1; 2.3 ± 1.1 of 5) exhibiting the highest and the domain pruritus (1.5 ± 0.9 of 5) and cognition (1.9 ± 0.9 of 5) the lowest scores. The markers of cholestatic liver injury ALP and gGT correlated with the PBC-40 domain social ($r = -0.270$, $p = 0.004$; $r = 0.194$, $p = 0.04$).

Conclusion The mean response rate to UDCA treatment in Germany at one year after treatment initiation and over a median time of 9 years is ~72%. The PBC-related symptom burden is high and non-response correlates with social concerns highlighting that symptoms in patients with PBC can be indicative of inadequate response.

2.27 Retrospektive Analyse des Therapieerfolgs von TIPS Anlagen und Identifikation prognostischer Parameter hierfür

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Die TIPS Anlage stellt eine minimal-invasive Technik dar über die der portalvenöse Druck effizient gesenkt werden kann und die daher bei Versagen konservativer Therapien von Komplikationen der portalen Hypertension eine wichtige Therapieoption darstellt. Ziel der vorliegenden Untersuchungen war die retrospektive Erfassung des Therapieerfolgs, sowie die Identifikationen potentieller Prädiktoren für ein Therapieansprechen.

Hierzu wurden sämtliche Patienten, bei denen am Universitätsklinikum Düsseldorf von 5.2005 bis 2.2017 eine TIPS Anlage erfolgte und die über mindestens 3 Monate ambulant nachbetreut wurden, erfasst. Insgesamt erfolgten in diesem Zeitraum 363 Neuanlagen eines TIPS. Hiervon wurden 191 Patienten für mindestens 3 Monate nachbeobachtet. Von diesen erfolgte bei 146 Patienten die Anlage zur Therapie eines refraktären Aszites, bei 45 Patienten zur Prophylaxe einer Rezidivblutung aus Varizen.

Bei der Gruppe die über 3 Monate nachbeobachtet werden konnte kam es nach TIPS Anlage innerhalb von 3 Monaten zu einem signifikanten Anstieg des Serumnatriums, der glomerulären Filtrationsrate, des Gesamteiweißes und des Albumins, sowie zu einem Abfall des Serumkreatinins, des Harnstoffs und des C reaktiven Proteins im Serum. Darüber hinaus kam es bei den Patienten, die über mehr als 12 bzw. 24 Monate nachbeobachtet wurden zu einer signifikanten Verbesserung des Child Pugh Scores. Eine effektive Therapie des Aszites konnte bei 73% (106 von 145) der Patienten erreicht werden, bei denen die Anlage aufgrund, von therapierefraktärem Aszites erfolgte. Bei 96% der Patienten, bei denen die Anlage aufgrund von Varizenblutung durchgeführt wurde, wurde eine Re-Blutung verhindert.

Als potentielle Prädiktoren für das Therapieansprechen bei Aszites konnte der Stentdurchmesser (p 0.001; OR 0,49), sowie die Konzentration des Harnstoffs im Serum (p 0,018; OR 0,98) ermittelt werden. Hinsichtlich des Therapieansprechens zur Prophylaxe von Varizenblutung konnte letztlich kein signifikanter Prädiktor ermittelt werden.

Übereinstimmend mit der Literatur belegen die Ergebnisse, dass die TIPS Anlage ein effizientes Verfahren zur Therapie von refraktärem Aszites und zur Prophylaxe von Varizenblutungen darstellt. Langfristig führt sie ferner zu einer signifikanten Verbesserung des Child Pugh Scores. Hinsichtlich des Therapieansprechens bei therapierefraktärem Aszites scheinen der Stentdurchmesser und die Höhe des Serumharnstoffes vor TIPS Anlage als prädiktive Parameter geeignet zu sein.

2.28 Epidemiologie und Auswirkungen bakterieller Infektionen bei stationären Patienten mit Leberzirrhose

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Hintergrund Bakterielle Infektionen spielen eine entscheidende Rolle bei der Morbidität und Mortalität von Patienten mit Leberzirrhose. Zur Prognoseverbesserung ist eine frühzeitige und zielgerichtete Therapie entscheidend. Prävalenzdaten zu bakteriellen Infektionen und dem assoziierten Erregerspektrums sind in diesem Kollektiv für den deutschen Raum mangelhaft. Ziel dieser Studie war es daher die Prävalenz bakterieller Infektionen und das assoziierte Erregerspektrum bei hospitalisierten Patienten mit Leberzirrhose an der Unimedizin Mainz zu untersuchen.

Methoden Alle Patienten mit Leberzirrhose, welche notfallmäßig zwischen März 2019 und August 2019 an der Universitätsmedizin Mainz hospitalisiert waren, wurden in diese prospektive Kohortenstudie eingeschlossen. Patienten mit einem HCC wurden nicht berücksichtigt. Jeder Patient wurde klinisch, laborchemisch und mikrobiologisch auf das Vorliegen einer Infektion untersucht. Zugrundeliegende Daten der Leberzirrhose, Infektionsdaten, die antibakterielle Therapie, sowie der weitere klinische Verlauf wurden erfasst.

Ergebnisse Insgesamt wurden 85 Patienten mit einem medianen Alter von 59 Jahren in die Studie eingeschlossen. 69,7% der Patienten waren Männer und die führende Ätiologie der Leberzirrhose war der chronische Alkoholkonsum (64%). Die Mehrzahl der Patienten wies eine fortgeschrittene Leberzirrhose auf (Child-Pugh B: 55%, Child-Pugh C: 30%). 47 Patienten (53,4%) litten bei Studieneinschluss an einem akut-auf-chronischem Leberversagen. 28,9% der Patienten litten bei stationärer Aufnahme an einer bakteriellen Infektion. Eine nosokomiale bakterielle Infektion trat im Mittel nach 12 Tagen (n = 28) auf. Die häufigsten Lokalisationen einer nosokomialen Infektion waren Pneumonien (32,1%) und Infektionen der Harnwege (32,1%). Erreger der Erstinfektion waren zumeist grampositiv (62,5%). Im Rahmen der Zweitinfektionen kamen gramnegative Erreger (46,7%) gehäufter vor. Ein drittel der Patienten (30,6%) verstarben im weiteren Verlauf. Die häufigste Todesursache waren bakterielle Infektionen (40,9%).

Diskussion Bakterielle Infektionen sind häufige Gründe für nicht-elektive Hospitalisierungen von Patienten mit Leberzirrhose. Das nachgewiesene Erregerspektrum wandelte sich bei Erstinfektion von einem vornehmlich grampositiven hin zu einem gramnegativem bei nosokomialen Infektionen. Die Wahl der antiinfektiven Therapie sollte hieran angepasst werden, um die Prognose betroffener Patienten zu verbessern.

2.29 Wertigkeit der Minilaparoskopie bei der makroskopischen Beurteilung der Lebersteatose

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Einleitung Die Wertigkeit der makroskopischen Beurteilung der Leberoberfläche bzgl. des Fibrosegrades im Rahmen einer Minilaparoskopie ist hinreichend bekannt als ein wichtiges diagnostisches und prognostisches Tool. Daten mit Beurteilung der Leberoberfläche bzgl. der Lebersteatose sind allerdings sehr rar.

Methoden In dieser retrospektiven unizentrischen Analyse wurden Daten von 212 Patienten mit einer nicht-viralen Hepatopathie erhoben mit Vorliegen von Ergebnissen zur minilaparoskopisch-makroskopischen, histologischen und sonographischen Beurteilung der Leber. Bezogen auf das Histologie-Ergebnise als Goldstandard wurde die Sensitivität/Spezifität/positiver und negativer prädektiver Wert der anderen zwei Untersuchungsmodalitäten evaluiert.

Ergebnisse Die sonographische Beurteilung der Leber erwies sich bezogen auf das Histologie-Ergebnis im Vergleich zur minilaparoskopischen Beurteilung der möglichen Lebersteatose (positive Xanthografie) als deutlich genauer. So zeigte sich die Sensitivität/Spezifität der Sonografie in Abhängigkeit von dem histologischen Grad der Lebersteatose bis zu 82,6%, bzw. 71,1% (histologisch Steatose >Grad II). Bei der minilaparoskopischen Beurteilung der Leber zeigte sich wiederum eine deutlich schlechtere Sensitivität/Spezifität mit 76,2%, bzw. 42,1%.

Schlussfolgerung Die Evaluation der Lebersteatose (Xanthografie positiv oder nicht) im Rahmen der minilaparoskopischen Untersuchung zeigt sich als ein weniger zuverlässiges Tool verglichen mit der sonographischen Beurteilung der Leberverfettung bei nicht-viralen Hepatopathien.

2.30 Dental health needs assessment and treatment in patients awaiting liver transplant

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Background and Aims Dental health is assessed in patients with liver cirrhosis awaiting liver transplant to avoid infectious complications due to hematogenous spread from dental foci after successful transplant. However, this recommendation relies on rather old studies. We investigated dental health in liver cirrhosis patients to clarify if dental care is necessary before transplant.

Methods Clinical and laboratory data from patients with liver cirrhosis who received a liver transplant between 2010 and 2018 in our department were retrospectively analysed for oral foci of infection in different patient subpopulations and for outcome after transplantation.

Results Data on oral health were available from 110/185 patients who received a liver transplant. Median age of patients was 53 years, 67% were male and the most frequent causes for liver transplant were alcoholic liver diseases (34%), viral hepatitis (28%) and autoimmune liver disease (14%). 35 patients showed good oral health without need for dental care, in 39 patients dental care was performed because of pretransplant assessment, 36 patients did not receive dental care despite poor oral health. The most common dental issues encountered were caries, which affected 75% of all patients, and periodontal diseases with a prevalence of 40%. We noted that need for dental care was highest in alcoholic liver disease (95%) compared to viral hepatitis (71%) and autoimmune liver disease (73%). Bleeding complications due to oral care occurred in 5/13 patients, who all presented with platelets below 70 G/L and an INR above 1.5. Mortality in the first 9 months after liver transplant was similar for patients with poor oral health, after dental care and without need for dental care (19%, 11% and 14%, respectively). However, the number of infections after transplant were higher in patients with poor oral health before transplant (2,9) compared to patients who received dental care (1,9) or patients with good oral health (1,8) ($p = 0.02$). In particular, infections by streptococci affected more frequently patients with poor oral health compared to patients without need for or after dental care (25% vs. 8%; $p = 0.02$).

Conclusion Because dental health was frequently compromised irrespective of aetiology of liver disease and because frequency of infections was lower after transplant in patients with good dental health, our data highlight that all patients awaiting liver transplant should be considered for dental care.

2.31 Correlation of Liver Perfusion with Outcome of Patients with Severe Liver Disease – A Prospective Cohort Study

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Chronic liver disease is one of the major causes of morbidity and mortality worldwide. Pathophysiologically, inflammatory processes lead to hepatocellular damage and fibrosis of liver tissue. Intrahepatic resistance increases, resulting in a change in the hemodynamics of hepatic blood vessels. The ultrasound examination plays an important role in the evaluation of liver diseases. However, the precise role and prognostic value of routine Doppler sonography of liver perfusion in patients with severe liver disease has not been sufficiently examined. The aim of this study is to determine a correlation of hepatic perfusion with outcome of patients with severe liver disease in the context of intensive care treatment.

Fifty patients hospitalized with severe liver disease in the internal intensive care unit of the Department of Internal Medicine I of the University Hospital Regensburg were routinely examined with sonography twice a week. To quantify liver perfusion, the hepatic artery resistance index (HARI) and the maximum portal vein velocity (PVv) were determined by means of Doppler ultrasound. At the time of the study, the MELD (Model for End-Stage Liver Disease) score was calculated for each patient. In addition, clinical data such as the current catecholamine dose, the mean arterial blood pressure, ventilation and laboratory parameters were collected. Finally, the duration of intensive care treatment and the outcome of the patients were also correlated with sonographic parameters.

By analyzing the obtained parameters of the MELD score, the HARI and the maximum PVv in a scatter plot a linear, positive correlation between HARI and MELD score and a linear, negative correlation between maximum PVv and MELD score were shown. Initial regression analyses quantify these correlations with a R2-value of 0,220 (HARI – MELD score) and 0.078 (PVv – MELD score). Furthermore, it appeared that the HARI in patients who died during inpatient treatment increased on average by 1.6% with each examination, whereas it declined by 0.3% in non-deceased patients. On average, with each examination the maximum PVv increased by 1.8% in deceased patients and by 16.5% in non-deceased study participants.

The correlation of HARI and maximal PVv with the MELD score shows that the development of liver perfusion is a prognostic factor. The routine assessment of HARI and the maximum PVv in patients with severe liver disease in the ICU should be further evaluated.

Poster Visit Session III Metabolism (incl. NAFLD) Friday, February 14, 2020, 4:40 pm – 5:25 pm, Lecture Hall P1

3.4 FOXA2 replaces FXR to maintain BSEP expression on bile canaliculi in acute-on-chronic liver failure

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Background & Aims The apical molecule BSEP is a key transporter in charge of bile acid delivery. In physiological conditions, nuclear receptor FXR controls BSEP expression by binding to its promoter. In acute-on-chronic liver failure (ACLF), systemic inflammatory response (SIRS) inhibits FXR expression or leads to loss of FXR nuclear translocation. Interestingly, most hepatocytes of ACLF patients maintain BSEP expression on bile canaliculi. The current study is investigating the regulatory mechanisms underlying BSEP expression in ACLF.

Methods We collected liver tissue from 15 ACLF patients. Among them, 10 received liver transplantation and 5 recovered spontaneously. BSEP and transcription factors FXR and FOXA2 were examined by immunohistochemistry. Hepatocellular polarity and BSEP expression regulation were investigated in mouse primary hepatocytes and AML 12 cells.

Results In the examined ACLF patients regardless of clinical outcome, most hepatocytes maintained BSEP expression on bile canaliculi, although their nuclei do not stain positive for FXR. Instead, BSEP positive hepatocytes robustly express FOXA2 that as well owns binding sites at BSEP promoter. In cultured hepatocytes, ectopic FOXA2 erexpression increases while FOXA2 knockdown reduces BSEP expression. ChIP analysis confirm direct FOXA2 binding to the BSEP promoter. Further, reduced BSEP expression from FXR depleted hepatocytes is completely restored by forced expression of FOXA2. FOXA2 expression and its subcellular localization is regulated by glucagon and insulin. Administration of glucagon to mouse hepatocytes induces FOXA2 expression, therewith maintaining BSEP on the bile canaliculi. On the other hand, insulin treatment remarkably inhibits nuclear translocation of FOXA2 in

hepatocytes. These *in vitro* findings are consistent with clinical observations: (1) The enrolled ACLF patients who express FOXA2 present with high glucagon levels due to SIRS associated high blood glucose. (2) SIRS-induced insulin resistance in liver facilitates FOXA2 nuclear translocation.

Conclusions Human body owns two different regulatory systems to respond to physiological or pathological circumstances for maintenance of BSEP on bile canaliculi. FXR controls BSEP expression in physiological conditions. In ACLF with depleted FXR function, FOXA2 maintains BSEP expression and canalicular localization in hepatocytes. FOXA2 expression and subcellular localization are controlled by glucagon and insulin, respectively.

3.5 Hepatic IL-1 signaling in NAFLD is a driver of whole-body insulin resistance and adipose tissue inflammation.

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Question There is emerging evidence supporting the concept that low-grade, chronic metabolic inflammation – to a large degree emanating from the hepatic compartment of patients with non-alcoholic fatty liver disease (NAFLD) – is a driving force of extrahepatic comorbidities. This study examines the role of hepatic interleukin (IL)-1 signaling as a trigger of systemic insulin resistance and inflammation.

Methods 8 – 10-week-old male hepatocyte-specific IL-1 receptor type 1 (IL-1R1) knockout mice (*Il1r1*$^{Hep-/-}$) and their wild-type (WT) littermates were fed a high-fat, high-carbohydrate diet (HFD) for 12 weeks to induce obesity-related NAFLD or a control diet.

Results All mice fed the HFD developed an obese phenotype – characterized by a significant weight gain and associated systemic metabolic alterations (hyperlipidemia, hyperglycemia and decreased adiponectin levels) – and an accompanying macrovesicular hepatic steatosis. Despite comparable metabolic stress induced by HFD, levels of serum transaminases, hepatic microvesicular steatosis and activation of c-Jun N-terminal kinases (JNK) and extracellular signal-regulated kinases (ERK) were significantly higher in the WT compared to *Il1r1*$^{Hep-/-}$ mice, suggesting a different pattern of liver injury and mitochondrial function. Moreover, *Il1r1*$^{Hep-/-}$ mice on HFD displayed an improvement of both hepatic and peripheral insulin sensitivity as evidenced by increases in hepatic insulin receptor expression and Akt (Ser473) phosphorylation, reduced fasting insulin and HOMA-IR levels and improved whole-body glucose tolerance following intraperitoneal glucose challenge. This was accompanied by reduced adipose tissue inflammation as reflected by decreased gene expression of F4/80, monocyte chemotactic protein (MCP)-1 and IL-1 receptor antagonist (IL-1RA) in *Il1r1*$^{Hep-/-}$ mice compared to WT mice.

Conclusions This data suggests a pivotal role of the IL-1R1 signaling pathway in hepatocytes in controlling whole-body insulin sensitivity and adipose tissue inflammation in the context of increasing metabolic burden.

3.6 Lipid droplet proteins in alcoholic and non-alcoholic steatohepatitis in patients with polymorphisms in PNPLA3

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Question Lipid droplets (LDs) are crucial organelles that play a central role in lipid homeostasis. A particular lipase associated with LDs is the patatin-like phospholipase domain containing protein 3 (PNPLA3). The I148 M single nucleotide polymorphism of PNPLA3 has been shown to be associated with non-alcoholic fatty liver disease and the development of hepatocellular carcinoma. Aim of the study was to investigate the underlying molecular mechanism driving the progression of the disease.

Methods To unravel the interplay between PNPLA3 and the LD-associated proteins of the perilipin-family in the regulation of LDs, (immuno)-histochemical analysis of a collective of 47 ASH- and 25 NASH-patients with known PNPLA3-status was undertaken. In addition, co-immunoprecipitation experiments were performed to identify novel PNPLA3 binding partners. Finally, the impact of the polymorphism on the lipolytic activity was determined.

Results Histologically, livers of I148 M carriers showed enhanced ballooning, acinar inflammation, microgranulomas and increased fibrosis with a prominent staining for perilipin 2 at ballooned hepatocytes. Perilipin 5 localized less to LDs, but showed a more cytoplasmic and partially nuclear localization instead. Interestingly, hepatocytes that were strongly positive for PNPLA3 showed diminished perilipin 1-expression.

Furthermore, we identified perilipin 5 and the lipase PNPLA2/ATGL as novel interaction partners of PNPLA3 that co-localized at LDs. In addition, we could show that perilipin 5, PNPLA3, and PNPLA2 are part of the same complex and that perilipin 5 is enhancing the binding of PNPLA3 with PNPLA2 dramatically. Strikingly, we could show dimerization of PNPLA2, a process inhibited by PNPLA3. Addressing the lipolytic activity of PNPLA2 revealed an inhibitory effect of PNPLA3, which is strongest for PNPLA3(I148 M).

Conclusion In summary, our data indicate that PNPLA3 regulates lipolysis by repressing the lipolytic activity of PNPLA2 in a perilipin 5-dependent manner most likely via disrupting active PNPLA2 homo-dimers/oligomers. The enzymatically inactive PNPLA3(I148 M) even further reduces PNPLA2-mediated lipolysis and increases toxic accumulation of lipids. We have thereby unraveled a mechanism of PNPLA3 in the progression of steatotic liver diseases. Concerning the long-standing debate on why and when bland steatosis progresses to steato-hepatitis, our data point to a critical step in lipolysis rather than in lipogenesis itself.

3.7 *Abcg8* 19 H Variante im Mausmodell: Größere Gallenblasenvolumina und gesteigerte biliäre Sterolkonzentrationen

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Hintergrund Gallensteine entstehen aus Cholesterinkristallen infolge biliärer Cholesterinübersättigung. Genomweite Assoziationsstudien identifizierten die 19 H-Variante des *ABCG8*-Gens als genetischen Risikofaktor. Der ABCG5/G8-Transporter kontrolliert in Hepatozyten und Enterozyten die Sekretion von Cholesterin und Phytosterinen in die Galle, bzw. das Darmlumen. Ziel der Studie war die phänotypische Charakterisierung von Mäusen mit homozygoter *Abcg8* 19 H Variante.

Methodik Mithilfe der BAC-basierten Rekombination wurden *Abcg8*-Knockin-Mäuse (KI) generiert, in deren *Abcg8*-Gen eine Base Guanin zu Cytosin verändert ist, was auf Proteinebene zum Austausch von Asparaginsäure gegen Histidin an Position 19 führt (D 19 H). Der KI-Phänotyp wurde makro- und mikroskopisch charakterisiert. Nach Anlage einer akuten Gallenfistel wurde die hepatische Galle gesammelt, und die neutralen Sterole in der Lebergalle und Leber wurden quantifiziert. Zusätzlich wurden Expressionsanalysen relevanter Gene des Cholesterinstoffwechsels durchgeführt.

Ergebnisse Die Gallenblasengalle war unabhängig vom Genotyp klar und ohne makroskopisch erkennbare Gallensteine. Die Gallenblasenvolumina nach 12-stündigem Fasten waren bei den weiblichen KI signifikant größer (42 ± 17 vs. 22 ± 7 µl), während bei männlichen KI kein wesentlicher Unterschied bestand. Die Galleflüssigkeit der weiblichen KI enthielt deutlich mehr Flüssigkristalle als die der gleichgeschlechtlichen Kontrollen. Die Cholesterinsekretion in die Galle war gesteigert (Chsec = (0,30 ± 0,10) + (0,07 ± 0,001)*GSsec vs. Chsec = (0,15 ± 0,07) + (0,03 ± 0,006)*GSsec). Der makroskopische Leberphänotyp und die hepatische Cholesterinkonzentration waren unauffällig bei den KI. Die relative Phytosterinkonzentration in der Leber war jedoch signifikant erhöht (22,8 ± 4,17 vs. 17,4 ± 4,62 mg/g Cholesterin; p = 0,0024). Srebp2 und die nachgeschalteten Gene Hmgcr, Ldlr und Pcsk9 waren in der Leber reprimiert. Cyp7a1, Tm6sf2 und Pnpla3 wurden ebenfalls vermindert exprimiert.
Schlussfolgerungen Der Phänotyp der Mutanten unterstützt die Assoziation der *Abcg8* 19 H Variante mit Cholesterinhypersekretion und liefert Hinweise für eine Dysfunktion des ABCG5/8-Transporters. Die Gallenblasenhypomotilität, welche sich in den größeren Gallenblasenvolumina widerspiegelt, könnte auf eine vermehrte Cholesterineinlagerung in die Gallenblasenwand zurückzuführen sein. Die Cholesterinhypersekretion trotz mutmaßlicher ABCG5/8-Dysfunktion lässt vermuten, dass ein ABCG5/8-unabhängiger Sekretionsweg existiert.

3.8 *PNPLA3* and *HSD17B13* gene variants exert opposite effects on fatty liver phenotypes: results from the FLAG cohort

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Background Non-alcoholic fatty liver disease (NAFLD) is a prevalent condition common in overweight and obese individuals. The *PNPLA3* p.I148 M variant represents a major genetic determinant of NAFLD progression. Recently the loss-of-function *HSD17B13* polymorphism rs72613567 was reported to be hepatoprotective in patients with chronic liver diseases (Abdul-Husn et al. *NEJM* 2018). Here we investigate the effects of the *PNPLA3* and *HSD17B13* variant on NAFLD phenotypes in a "real life" cohort of German NAFLD patients.
Patients and methods All patients were recruited within the Fatty Liver Assessment in Germany (FLAG) program, a multicenter cohort study covering private and public outpatient clinics. The *PNPLA3* p.I148 M and the *HSD17B13* rs72613567 polymorphisms were genotyped using allelic discrimination assays. The control cohort comprises 174 healthy individuals. The effects of both variants were analysed in contingency tables and regression analyses.
Results Overall, the study cohort comprised 475 individuals (255 men) with NAFLD. The *PNPLA3*, but not the *HSD17B13*, polymorphism deviated significantly (P < 0.001) from Hardy-Weinberg equilibrium (HWE) in the entire FLAG cohort due to overrepresentation of the prosteatotic risk allele. The *PNPLA3* p.I148 M variant was more prevalent among FLAG patients as compared to healthy controls and increased the risk of developing NAFLD (common OR = 2.47, P = 5 × 10 – 09). It also correlated with serum AST (P = 0.04) and ALT (P = 0.01) activities. Notably, among carriers of the *PNPLA3* p. 148 M allele, presence of the *HSD17B13* allele was associated with lower AST (P = 0.006) and ALT (P = 0.002) activities, underscoring the protective effects of this variant. Finally, the *PNPLA3* p.I148 M polymorphism was associated with an increased risk of presenting with liver stiffness ≥ 9.2 kPa (common OR = 1.50, P = 0.03), i. e. with significant fibrosis (Caballería et al. *Clin Gastroenterol Hepatol* 2018*)* and this association remained significant (P = 0.04) in a multivariate model including the *HSD17B13* polymorphism.
Discussion Previous genetic studies in NAFLD patients were mostly performed in tertiary academic referral centres. Here, by analysing patients from a "real life" NAFLD cohort, we further underscore the role of the *PNPLA3* variant as the central genetic trigger and modulator of NAFLD. We also demonstrate that the *HSD17B13* polymorphism can attenuate some of the harmful *PNPLA3*-associated effects.

3.9 Metabolic reprogramming in livers of mice with chronic liver disease

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Metabolic zonation of healthy liver lobules is extensively studied. However, little is known how chronic liver disease (CLD) influences lobular zonation. To bridge this gap, we studied metabolic zonation in two mouse models of CLD: repeated intoxication with carbon tetrachloride (CCl4; 1 gram/kg) twice a week up to one year, and bile duct ligation (BDL) for 21 days. Disease progression and its impact on metabolic zonation was analysed by histopathology, immunohistochemistry, image analyses, RT-PCR, as well as RNA-sequencing (RNA-seq) techniques.
Repeated CCl4 intoxication triggered centro-centro bridging pattern of fibrosis that was detected already at 2 months. At late stages (1 year), progression to cirrhosis with presence of regenerative and neoplastic nodules was detected. RNA-seq analysis revealed downregulation of the pericentral and upregulation of the periportal genetic programs, respectively. Furthermore, immunohistochemistry analysis revealed spatio-temporal alterations of the pericentral and the periportal proteins. At the early stage (2 – 6 months), the pericentral proteins, e. g. CYP450 enzymes and glutamine synthetase, showed decreased diameter of the positive area and centro-centro bridging pattern. At the late stage (1 year), the expression of the pericentral proteins was almost completely lost. In contrast, the territory of the periportal proteins, e. g. the urea cycle enzymes, was increased time-dependently to cover almost the entire liver parenchyma. To check whether the loss of pericentral gene expression occurs because CCl4 intoxication targets the pericentral hepatocytes, the same analyses was done after BDL; a model of periportal fibrosis. Interestingly, the pattern of periportalization of the liver lobule was also con-

sistent in the BDL model. Biostatistical analysis of the RNA-seq data suggested that periportalization of the liver lobule in CLD was due to a loss of Wnt/β-catenin signalling pathway. In order to check the functional consequences of this altered zonation, mice on day 21 post BDL or on one year of repeated CCl4 intoxication were challenged with 200 mg/kg acetaminophen (APAP). Interestingly, both mouse models were almost completely resistant to APAP intoxication.

In conclusion, CLD strongly alters zonation of the liver lobule where the periportal genetic program becomes dominant, forming a "periportal-like" lobule.

3.10 Lipid-loaded Hepatocytes fail to Suppress IL-4 Production by Human CD4+ iNKT Cells

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Background Invariant natural killer T (iNKT) cells recognising glycolipids presented by CD1d are implicated in the inflammatory cascade associated with hepatic steatosis and its progression to steato hepatitis (NASH). Therefore, we established an in vitro steatosis model of HepaRG cells to elucidate the contribution of iNKT cells to initiation of inflammation after steatosis.

Methods Deposition of 0.5 mM free fatty acid (FFA) on HepaRG was achieved by treating the cells with 2:1 oleic acid and palmitic acid for 24 hours. Next, the effect of steatosis on iNKT cells was investigated by differentiating freshly isolated iNKT cells with FFA-loaded HepaRG for 7 days. Thereafter, cytokine profile and activation status of differentiated iNKT cells was analyzed by flow cytometry.

Results The direct coculture of iNKT and HepaRG prevented the spontaneous production of IL-4 in differentiated iNKT cells (HepaRG vs. medium alone: 16% vs. 31 %, $p = 0.02$). However, the presence of FFA in HepaRG significantly affected this suppressive effect (HepaRG vs. FFA-HepaRG 16% vs. 20%, $p = 0.03$). Furthermore, expansion of iNKT cells in transwell separated from HepaRG indicated that this suppressive effect requires a direct contact between both cells (HepaRG vs. HepaRG+TW: 16% vs. 33%, $p = 0.004$and FFA-HepaRG vs. FFA-HepaRG+TW: 20% vs. 34%; $p = 0.04$). Luminex analysis of the coculture supernatants revealed expression of IL-15 by hepatocytes in contact with iNKT, which was hijacked by the iNKT cells to promote the expansion of IL-4+population. Nevertheless, the unloaded control employed an unknown mechanism disrupted by FFA accumulation to maintain the suppression of IL-4 + iNKT even in the presence of IL-15. Finally, analysis of intrahepatic and peripheral iNKTs from patients with and without steatosis indicated a higher activation state of iNKT (Fatty vs. non-fatty PBL HLA-DR+iNKT: 30% vs. 15%, $p = 0.0001$ and IHL HLA-DR+ iNKT: 23% vs. 10%, $p = 0.013$) and frequency of IL-4+PBL iNKT (Fatty vs. non-fatty PBL IL-4+iNKT: 13% vs. 28%, $p = 0.038$) in patients with steatosis.

Conclusion In summary, the results show that IL-4+iNKT might contribute to inflammation in hepatic steatosis because a suppressive mechanism of liver cells is compromised by fat accumulation.

3.11 Intestinal deletion of fatty acid transport protein 4 in mice increases blood chylomicrons

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Polymorphisms of fatty acid transport protein 4 (FATP4) are associated with blood lipoproteins and insulin resistance. As FATP4 is localized in intracellular organelles and highly expressed in mouse small intestine, Fatp4 role in fat absorption in mice is still controversial. As our recent studies have suggested Fatp4 role in triglyceride (TAG) metabolism, we here investigated intestinal fat absorption and blood TAG-chylomicrons (CM) in villin-Cre specific inactivation of the Fatp4 gene with exon 3 deletion (entFatp4KO mice).

Methods Blood lipoproteins were measured by using gel-permeation high-performance liquid chromatography. Lipidomics were carried out using ESI/MS-MS and LC/MS-MS.

Results Under chow feeding, entFatp4KO showed no difference in villous and crypt architecture but showed a decrease of intestinal very long chain fatty acids, phospholipids (PL), sphingomyelin and ceramides (Cer) concomitant with an increase of cholesterol esters (CE). Following overnight starvation, male entFatp4KO showed an increase of serum total TAG-rich and cholesterol (CHOL)-rich lipoprtoeins (combined CM, VLDL, LDL, HDL) and particle numbers of CM and HDL by 20 – 30%. This was concomitant with an increase trend of serum glycerol and jejunum TAG. Following starvation and an oral gavage with 400 µl 2% intralipids for 4 h, female entFatp4KO also showed an increase of total TAG-rich lipoproteins and CM particle numbers by 30%. Following feeding with high-fat/high-CHOL diet (HFHC, 15% fat, 1.25% CHOL) for 16 weeks, female entFatp4KO showed an increase of body, liver, and spleen weights without altering liver enzyme activities. HFHC-fed entFatp4KO also showed marked increase of serum TAG, non-esterified free fatty acids, and PL as well as TAG-rich CM, VLDL, LDL, and glycerol by 60 – 80%. This was concomitant with a decrease of intestinal Cer and an increase of oleate-containing CE. Notably, these mutant mice showed a significant shift of ileal fat-droplet size from 20 – 30 towards 50 – 70 µm. This was concomitant with an increase trend of liver TAG but a significant increase of hepatocyte nucleus/cell diameter ratio.

Conclusions Intestinal Fatp4 deletion induced an increase in blood CM by diverting fatty acids from the syntheses of PL and Cer towards neutral lipids TAG and CE resulting in larger fat-droplet sizes after high-fat feeding. Our results implicate hyperchylomicronemia and hepatic abnormalities in patients with FATP4 mutations who consume high dietary fat.

3.12 Deletion of fatty acid transport protein 4 in HepG2 cells increases lipolysis lipids and lipoprotein secretion

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Expression of fatty acid transport protein 4 (FATP4) is increased in adipose tissues of individuals with central and acquired obesity, and polymorphisms of FATP4 are associated with blood lipids and insulin resistance. We have reported an increase of blood triglycerides (TAG), glycerol, and non-esterified free fatty acids (NEFA) in liver-specific Fatp4-deficient mice only when fed with high-fat diet. Here we investigated whether FATP4 plays a role in hepatic TAG metabolism in HepG2 cells. **Methods** Genetic deletion of FATP4 was performed by using CRISPR/Cas9 technology. To study FATP4 effects on hyperlipidemia, control (HepCon) and FATP4-knockout (HepFATP4KO) cells were treated with 300 or 600 µM oleate for 2 or 4 h. We studied TAG synthesis by using isotope labelled glycerol ($^{13}C_3$, 99%; D_8, 98%) and 13 species of $^{13}C_3D_5$TAG containing various fatty acids were probed in cells treated with 20 µM $^{13}C_3D_8$-glycerol for 2 h. **Results** Compared to HepCon, HepFATP4KO cells showed an increase of cellular $^{13}C_3D_5$TAG containing saturated and monounsaturated fatty acids (MUFA) under basal conditions. Interestingly, oleate-treated KO cells compared to treated HepCon showed an increase of $^{13}C_3D_5$TAG containing a combination of 18:2, 18:3 or 20:4, indicating a diversion of polyunsaturated fatty acids (PUFA) towards TAG. HepFATP4KO cells

under basal conditions also showed a marked increase of released VLDL and HDL as well as lipoprotein transport *MTTP* mRNA expression, all of which were not further altered by oleate treatment. Gene analyses revealed that HepFATP4KO cells showed downregulation of β-oxidation *CPT1B* and fatty-acid uptake *CD36*, but upregulation of lipolysis hormone-sensitive-lipase, all of which were not affected by oleate treatment. In support of lipolysis activity, HepFATP4KO cells at basal conditions showed an increase of glycerol and NEFA levels in both cells and supernatants, and the levels of these lipids together with TAG were further increased upon oleate treatment. Taken together, FATP4 deletion in HepG2 cells under basal conditions elicited fatty-acid trafficking towards synthesis of saturated and MUFA TAG leading to an increase of released lipoproteins and lipolysis lipids. Upon oleate treatment, FATP4 deletion preferentially caused an increase of PUFA TAG and lipolysis lipids. Our results implicate the role of FATP4 on hyperlipidemia by modulating fatty-acyl chain saturation in hepatocyte TAG.

3.13 Activation of the arylhydrocarbon receptor sensitizes to acetaminophen-induced acute liver failure

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Question Acetaminophen (APAP)- induced liver damage is one of the most common causes of acute liver failure. However, the risk factors determining hypersensitivity to APAP remain poorly defined. The transcription factor arylhydrocarbon receptor (AhR), which can be activated by environmental, dietary, microbial and metabolic ligands, is a direct inducer of CYP1A2, one of the major enzymes involved in the generation of toxic APAP metabolites. Therefore, we hypothesized that AhR activation might contribute to APAP-induced hepatotoxicity.

Methods Wildtype or conditional AhR knockout mice lacking AhR in hepatocytes (AlbΔ/ΔAhR) were subjected to a combined treatment with APAP and the non-toxic gut-derived AhR ligand 2-(1'H-indole-3'-carbonyl)-thiazole-4-carboxylic acid methyl ester (ITE).

Results Administration of ITE together with a normally sublethal APAP overdose (350 mg/kg) caused 80% mortality within 8 hours, whereas all vehicle-treated control mice survived ($p = 0.0002$). Accordingly, we found vast necrotic areas in liver tissue ($p < 0.0001$) and strongly elevated serum transaminase levels (ALT: 7616 vs. 812 U/L, $p < 0.0001$) in mice, which were co-treated with ITE and APAP, as compared to controls receiving vehicle and APAP. Of note, even at APAP doses equivalent to the recommended therapeutic range in humans, AhR activation by ITE induced substantial liver damage as compared to vehicle treatment (ALT: 1488 vs. 117 U/L, $p = 0.0043$). In contrast, AlbΔ/ΔAhR mice were largely protected from ITE-induced disease aggravation. Indeed, serum ALT levels (1063 vs. 3247 U/L), $p = 0.0012$) were significantly lower as compared to littermate controls. Moreover, TUNEL staining of liver sections revealed greatly reduced hepatocyte death in AlbΔ/ΔAhR mice following combined APAP and ITE treatment ($p = 0.007$). Mechanistically, AhR activation by ITE fueled hepatic accumulation of toxic APAP metabolites by up-regulating expression of the APAP-metabolizing enzymes CYP1A2 ($p < 0.0001$) and CYP2E1 ($p = 0.0077$), and of the glutathione-degrading enzyme gamma-glutamyltransferase (GGT) ($p = 0.0001$).

Conclusion AhR activation in hepatocytes induces accumulation of toxic APAP metabolites aggravating APAP hepatotoxicity. Thus, AhR activating ligands, which can originate from various sources, such as nutrition or gut microbiota, might be a risk factor sensitizing to APAP-induced acute liver failure.

3.14 Evaluation of a piglet model for Parenteral Nutrition-associated Cholestasis (PNAC)

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Total parenteral nutrition (TPN) is associated with the development of parenteral nutrition-associated liver disease (PNALD) in infants. Parenteral Nutrition-associated Cholestasis (PNAC) is one of the most common metabolic problems associated with TPN. PNAC can be a life threatening complication of TPN in newborn children, and might ultimately results in the need for a liver transplantation. Incidence of PNALD in infants who receive TPN for at least 2 month can be at 50%. Risk factors appear to be multifactorial including immature hepatic function or lack of enteral feeding, and not fully elucidated. Therefore we aimed to establish a piglet model to evaluate the development and progress of PNALD/PNAC, analyze the molecular mechanisms and compare the findings to human PNALD.

It has been shown previously that piglets at birth correspond to preterm infants regarding the developmental stage of their gastrointestinal tract and therefore we choose 3 day old piglets and divided them into two groups. The TPN group (TPN) underwent general surgery to place a central catheter in the external jugular vein followed by application of parenteral nutrition solution (liquid and lipid phase) adjusted to body weight and nutritional needs for 21 d. The control group (K) received formula for infant piglets orally for the same time period. Blood samples were taken during the experiment and on the final day to analyze liver enzymes (ALT, AST, GGT) and functional liver parameters (bilirubin, cholesterol, albumin, triglycerides). Tissue samples of liver, gallbladder, intestine, pancreas, spleen, lung, brain, muscle and fat were either snap frozen in liquid nitrogen or fixed in paraformaldehyde for histochemical analysis. Haematoxylin eosin staining was performed to evaluate cellular structure as well as sirius red staining for elastic or collagenous fibres. Grade of steatosis was determined by oil red O staining. Expression of specific mRNAs of inflammation (TNF-alpha, MCP1, IL 1β), fibrosis (TGFβ, ACTA2, COLα1, TIMP 1) and fat metabolism were quantified by qRT-PCR. Furthermore a score for cholestasis based on bile acid serum levels and histochemical as well as electron microscopy examination was established to evaluate degree and progress of PNAC.

3.15 Role of GPNMB in hepatic steatogenesis and cancer

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NAFLD is characterized by lipid deposition in liver cells and is progressed to NASH and HCC. Hepatocyte (HC)-derived Glycoprotein non-metastatic melanoma B (Gpnmb) was recently reported as a regulator of fat metabolism in adipose tissue, however, its role in the pathogenesis of NASH-related HCC are not clear. We aim to functionally investigate Gpnmb during liver steatogenesis and HCC. A NASH-based HCC mouse model, STAM was selected. In this model, NASH and HCC stages were analyzed. We also investigated publically available patient cohorts i.e. GSE48452 (NASH) and GSE14520 (HCC) datasets. An in vitro steatosis model was induced by oleic acid (OA) in primary mouse hepatocytes (PMHC) and AML-12. GPNMB expression was manipulated by overexpression (OE) and knockdown (KD) approaches. Steatosis induction and lipid metabolic targets was assessed upon modulation of GPNMB by PCR. Cell growth and death of Huh7 cells was analysed by MTT and caspase-3 assay, PCR, WB and time-lapse imaging. Comparative analysis of patient cohorts and

STAM mouse model identifies GPNMB as a consistently upregulated gene. IHC staining shows that GPNMB protein localizes in HC in healthy and NASH, and in HC-derived tumour cells in HCC tissues. In vitro, OA induces GPNMB in PMHC and AML-12 cells. Lipid accumulation increased upon KD of Gpnmb, and decreased by OE as measured by triglyceride (TG) level in OA-treated pMHC and AML-12 cells. We analyzed GPNMB dependent gene expression alterations of critical players in hepatic lipogenesis i.e. SREBP-1c, PPAR-α, PPARγ, Fasn and Scd1, as well as targets involved in FA oxidation as Cpt1 and Acox1. In line with TG accumulation, Gpnmb KD increases SREBP-1c, PPAR-α, PPARγ, Fasn and Scd1 mRNA expression in OA-treated PMHC and AML 12. However, Cpt1 and Acox1 are also upregulated. In case of GPNMB OE, we obtained complementary results. Preliminary data using Huh7 cells indicate that GPNMB OE suppresses cell proliferation and induces apoptosis. Mechanistically, GPNMB OE facilitates cell death via inhibition of AKT phosphorylation. GPNMB is a consistently upregulated target in NASH and HCC. In contrast to previous reports, GPNMB is expressed in HC and HC-derived cancer cells, instead of macrophages. In fatty liver, GPNMB is upregulated to tone down lipogenesis, therefore, it seems that GPNMB has a protective role against liver fat toxicity. In liver cancer cells, GPNMB acts as a tumor suppressor by providing cytostatic effects.

3.16 M30 als Biomarker für die nicht-invasive NAFLD-Diagnostik bei Patienten mit morbider Adipositas nach Roux-y-Magenbypass

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Einleitung Der Apoptosemarker M30 erhöht die Genauigkeit der nicht-invasiven Risikostratifizierung der NAFLD.[1] In Deutschland steigt die Zahl der Patienten mit morbider Adipositas, die u. a. aufgrund einer NASH einer bariatrischen Operation zugeführt werden, stetig an.[2] Das Ziel der vorliegenden Studie war die Validierung der Effekte einer Magenbypass-OP auf die Leber mit nicht-invasiven Massnahmen.

Methoden 39 Patienten mit Magen-Bypass wurden prä- und postoperativ auf Routineparameter, Fibrose-Scores und den Apoptosemarker M30 hin untersucht (T0: präoperativ, T1: bis 5 d, T2: bis 6 Wochen, T3: bis 3 Monate, T4: 6 Monate und T5: 12 Monate postoperativ). Zeitliche Verläufe und Korrelationen zwischen den verschiedenen Parametern wurden mittels einfaktorieller ANOVA und posthoc-Tukey Test und durch Korrelationen nach Pearson analysiert.

Ergebnisse Neben einem durchschnittlichen BMI-Abfall von 51,9 kg/m^2 auf 32,6 kg/m^2 innerhalb eines Jahres sanken die Serum-Triglyceride, LDL und HbA1c signifikant von oberhalb der Referenzwerte auf Normwertigkeit ab. Das HDL stieg signifikant an. Es zeigte sich direkt postoperativ sowohl eine systemische Inflammation als auch eine Verschlechterung der Leberwerte (ALT/AST). Durch Gruppenbildung mittels M30 lassen sich zwei Gruppen identifizieren. Gruppe A, die einen Abfall, der M30-Werte von über 300 U/L unter 160 U/L zeigt und Gruppe B, die im Vergleich zum Ausgangswerte eine Erhöhung der M30-Werte zeigt. Gruppe A zeigt einen signifikanten Abfall des APRI und der Leberwerte, während dies bei Gruppe B nicht nachgewiesen werden kann.

Schlussfolgerung Die Magen-Bypass-Operation wirkt sich bei 77% (30/39) der Patienten positiv auf die Leberwerte und bei allen positiv auf HDL-, LDL- und Blutglucose-spiegel aus. 23% der Patienten (9/39) zeigen sowohl eine transiente Verschlechterung als auch fehlende Besserung der hepatologischen Parameter nach einem Jahr Follow-up. Mittels nicht-invasiven Screening sollten jene Patienten, die der zweiten Gruppe angehören, hepatologisch nachverfolgt werden.

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3.17 Beneficial effects of high BMP-9 levels under diabetic and high fat conditions

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Bone morphogenetic protein (BMP)-9, a member of the TGFbeta family of cytokines is constantly produced in the liver and circulates in the body in an active conformation. The potential functions of BMP-9 are diverse and include regulation of liver homeostasis as well as fibrogenesis, inflammation or angiogenesis. The published findings that the serum levels of BMP-9 are reduced in newly diagnosed diabetic patients and that BMP-9 overexpression ameliorated steatosis in high fat diet induced obese mice, prompted us to further investigate the role of BMP-9 in conditions of steatosis and/or diabetes. We followed the hypothesis that BMP-9 might act similar as insulin itself in diabetic conditions and that via gut-liver-cross-talk BMP-9 might be protective against high-fat diet induced steatosis. BMP-9 KO mice (on a C57/Bl6 background) appeared rather over-weight with partially massive visceral fat deposition whereas the livers appeared quite normal and were rather small. This resulted in a significantly decreased liver to body weight ratio in these mice. To reproduce published data obtained with human serum from diabetic patients, we analyzed the blood of STZ rats (animal model for diabetes type I) and could confirm that BMP-9 levels are reduced. However, when looking at the RNA levels of the livers from these rats by real-time PCR we did not find any reduced BMP-9 expression in the diabetic animals. This implies that the basal amounts of BMP-9 in the serum have already been used up but without a subsequent, compensatory upregulation of hepatic expression. Using human gut organoids we additionally found that BMP-9 upregulates expression of Fgf19, a factor that was described to protect the liver from steatosis. This correlated well with enhanced expression of the FGF19 receptor beta-Klotho in BMP-9 stimulated human hepatocytes.In summary these data support the conclusion that under high sugar and/or high fat conditions increased levels of BMP-9 would most likely be beneficial but with insulin resistance or lack of insulin such upregulation may not occur any more, making supplementation of BMP-9 also an interesting approach for future therapies.

3.18 Fibroblast growth factor 21 response in a preclinical alcohol model of acute-on-chronic liver injury

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Background and Aims Fibroblast growth factor (FGF) 21, the major regulator of glucose and lipid homeostasis, has been shown to have a potential role in bile acid metabolism. We aim to investigate the FGF21 response in an acute-on-chronic liver injury (ACLI) model in *Abcb4*-/- mice with chronic hepatobiliary injury, where ethanol challenge was used as acute trigger.

Method Total RNA was extracted from wild-type (WT) C57BL/6J and *Abcb4-/-* (KO) mice, which were either fed control diet (WT/Cont and KO/Cont groups) or ethanol diet (5% v/v), followed by an acute ethanol binge (5 mg/kg) (WT/EtOH and KO/EtOH groups; n = 28/group). Hepatic expressions of *Fgf21*, *Fgfr1*, *Fgfr4*, *Klb*, *Srebf1*, *Cyp7a1*, *Cyp8b1*, *Cyp27a1*, *Shp*, *Fxr*, *Ppara* and *Mtor* as well as ileal mRNA levels of *Fgf15*, *Fgfr1*, *Klb*, *Fxr* and *Diet1* were evaluated using the 2-ΔΔCt method. Plasma FGF15 and FGF21 levels were determined by ELISA. ANOVA was performed for statistics.

Results *Fgf21* was significantly upregulated after ethanol exposure in WT and KO mice (p = 0.009 and 0.026, respectively) compared to their control diet fed counterparts. FGF21 elevation was observed in plasma of WT/EtOH and KO/EtOH groups (p = 0.040 and 0.048, respectively). Hepatic expressions of *Fgfr1, Fgfr4, Klb, Srebf1, Shp, Fxr* and *Mtor* showed no difference between groups. *Cyp7a1* and *Cyp27a1* were significantly repressed in livers of WT/EtOH (p = 0.044 and p = 0.007, respectively) and KO/EtOH groups (p = 0.035 and p = 0.001, respectively), compared to WT/Cont. Ethanol challenge resulted in significant induction of hepatic *Ppara* expression in both WT (p = 0.021) and KO mice (p = 0.023). Significant repression of *Cyp8b1* was observed only in KO/EtOH group, compared to WT/Cont (p = 0.041), WT/EtOH (p = 0.029) and KO/EtOH groups (p = 0.027). While plasma FGF15 levels were not different between groups, ileal expressions of *Fgf15* and *Fxr* were upregulated in WT/EtOH compared to WT/Cont mice (p = 0.029 and p = 0.007, respectively). Ileal *Diet1* expression did not differ.

Conclusions Alcohol consumption markedly suppressed hepatic expression level of *Cyp7a1*, which encodes the rate-limiting enzyme of bile acid synthesis, regardless of the genotype. Simultaneous upregulation of *Fgf21* and downregulation of *Cyp7a1* in liver, together with the invariant plasma FGF15 and hepatic *Shp, Fxr, Klb, Fgfr4* levels, suggest that upon ethanol challenge, bile acid metabolism may be regulated by FGF21, resulting in an inhibition of CYP7A1 through an FGF15-independent pathway in our model.

3.19 H_2O_2, the major reactive oxygen species produced during alcohol metabolism induces autophagy without involving mTOR

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Background and Aims Alcohol-mediated reactive oxygen species (ROS) formation in the liver, mainly H_2O_2, contributes to disease progression and eventually hepatocellular carcinoma development in patients with ALD. Enhancement or activation of autophagy, with the suppression of mTOR signaling, is likely to play an important role in early stages of the alcoholic liver disease (ALD). However, with the progression of the disease, the expression of mTOR increases dramatically leading to the suppression of autophagy. According to recent literature, this is accompanied with significant hepatic lipid accumulation and iron deposition as well as inflammation under persistent alcohol exposure. It is also known, that H_2O_2 is involved in the regulation of autophagy in both acute and chronic ALD models, however, the exact underlying molecular mechanisms are still unclear. Therefore, we investigated in vitro and in vivo by using alcohol mouse model alterations in mTOR signaling as well as downstream effects induced by H_2O_2 and low oxygen tension.

Methods Huh7 cells were cultured with the GOX/CAT system, which allows an independent control of hydrogen peroxide as well as oxygen levels, in combination with different doses of ethanol. LC3B, p62, mTOR and autophagy related proteins were analyzed by western blot. Same analyses were performed in liver tissues of C57BL/6 mice treated with acute (alcohol binge) and chronic ethanol (20% ethanol in the drinking water) for 4 weeks (n = 4).

Results H_2O_2 significantly increased LC3B activation and this effect could be efficiently blocked by N-acetyl cysteine (NAC), which is a ROS scavenger. Interestingly, even though the LC3B activation was increased by H_2O_2, the m-TOR expression was not suppressed as normally expected. Co-treatment of cells with H_2O_2 and the mTOR inhibitor Rapamycin led to an increased autophagic flux as compared to single treatment. The in vivo experiments showed a combined activation of LC3B and suppressed p62 and AKT levels as well as enhanced p-AMPK expression in the livers of the acute alcohol group. In contrast, mice exposed to chronic alcohol showed blocked autophagic flux with dramatically increased LC3-I and p62 levels.

Conclusion Our findings underscore an important role of H_2O_2 in regulating autophagy during acute and chronic alcohol ALD exposure. Further studies will be needed to to identify H_2O_2-induced signaling pathways that regulate autophagy.

3.20 Metabolische Veränderungen bei Erwachsenen mit homozygotem Alpha1-Antitrypsinmangel (Pi*ZZ Genotyp)

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Hintergrund Alpha1-Antitrypsin (AAT) ist ein hepatisch produziertes Protein mit zahlreichen metabolischen und immunomodulatorischen Funktionen. Der durch AAT-Mutation hervorgerufene, klassische schwere AAT-Mangel (Pi*ZZ) führt durch eine Leber- und Lungenbeteiligung zu einer verminderten Lebenserwartung. Da die metabolischen Auswirkungen dieser Mutation weitgehend unbekannt sind, evaluierten wir die Körperzusammensetzung und metabolische Parameter in einer großen, internationalen Kohorte von Pi*ZZ-Individuen.

Methodik Wir rekrutierten prospektiv 160 Pi*ZZ-Probanden und 80 gematchte Kontrollen ohne eine AAT-Mutation (Pi*MM). Lungenbeschwerden wurden anhand des COPD Assessment Tests (CAT) quantifiziert, wohingegen die Leberbeteiligung mittels transienter Elastografie (TE) inklusive liver stiffness measurement (LSM, Fibrose-Marker) und controlled attenuation parameter (CAP, Steatose-Marker) charakterisiert wurde. Die Körperzusammensetzung wurde mithilfe einer Bioimpedanz-Analyse (BIA) erfasst. Das Hepatokin Fetuin-A wurde mittels ELISA gemessen.

Ergebnisse Verglichen mit Pi*MM, präsentierten sich Pi*ZZ-Träger mit höheren CAT- (15,7 vs. 7,3, p < 0,0001), LSM- (7,3 vs. 4,3 kPa, p < 0,0001) und CAP-Werten (265 vs. 236 dB/m, p < 0,0001). Zudem waren sie stärker insulinresistent (HOMA-IR 2,74 vs. 1,61, p < 0,0001), hatten erhöhte Fetuin-A- (1,3 vs. 1,1 g/l, p < 0,0001) und erniedrigte Triglyzerid-Werte (95,83 vs. 124,34 mg/dl, p < 0,0001). Trotz ähnlicher BMI-Werte (24,53 vs. 24,89 kg/m2, p = 0,558), zeigten Pi*ZZ-Individuen weniger Skelettmuskelmasse (22,53 vs. 25,62 kg, p = 0,026) und einen erniedrigten Phasenwinkel, welcher als genereller Gesundheits- und Ernährungsmarker dient (5,29 vs. 6,01 °, p < 0,0001). Pi*ZZ-Patienten mit einer fortgeschrittenen Leberfibrose verfügten über ein erhöhtes viszerales Fett (VAT 3,31 vs. 2,28 Liter, p = 0,015), während Pi*ZZ-Individuen mit einer Lungenbeteiligung eine Sarkopenie aufwiesen (Skelettmuskelmasse 21,54 vs. 25,62 kg, p = 0,006). Die stärkste Korrelation mit LSM zeigte VAT (rho = 0,537, p < 0,0001); während VAT, Taillenumfang und BMI moderat mit dem CAP korrelierten.

Fazit Unsere Daten demonstrieren die in Pi*ZZ-Patienten stattfindenden metabolischen Veränderungen und weisen auf die Notwendigkeit einer ernährungsmedizinischen Betreuung dieser Patienten hin.

3.21 Progression of NAFLD and NASH is mediated by proinflammatory tissue-resident T cells

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Background The occurrence of non-alcoholic fatty liver disease (NAFLD) is closely associated with obesity and metabolic syndrome and is the leading cause of chronic liver disease in developed countries. Most often, hepatic steatosis leads to inflammation and metabolic dysfunction, which are the hallmarks of non-alcoholic steatohepatitis (NASH). Pathological immune responses are thought to be the main drivers of NAFLD progression but the mechanisms of pathogenesis remain incompletely understood. Therefore we aim to characterize the immunologic parameters that are involved in the progression of NAFLD.

Methods We collected PBMCs liver and mesenteric fat tissue samples from obese patients with and without NAFLD and/or NASH after informed consent. Biopsies were taken during bariatric surgery and patients were subsequently monitored for 12 months. 58 patients were included in the study (obese without NAFLD [n = 12], patients with NAFLD without inflammation [n = 28] and patients with NASH [n = 15]). Mediators involved in local immune responses were characterized by single cell RNA sequencing (scRNAseq) of CD3 T cells in liver and mesenterial fat tissue of two patients and subsequently validated by flow cytometry.

Results Thirteen disparate T cell subtypes were identified by scRNAseq. Effector CD8 T cells, MAIT cells and activated γδ T cells seemed to be enriched within the diseased tissue. CD8 T cells were accumulated within hepatic tissue whereas CD4 T cells were predominantly found in the fat tissue. Subsequent flow cytometric analysis revealed similar results. Furthermore, natural killer T (NKT) cells and NK cells were highly abundant in the hepatic tissue. We further elucidated the distribution of cytotoxic granules within tissue cells and circulation more deeply. Cytotoxic granules containing perforin, granulysin and granzymes were predominantly found within the CD8 T cell population. The majority of cytotoxic granules were detected within subpopulations of innate immune cells (especially NKT cells, NK cells and γδ T cells). Moreover, peripheral blood NKT cells and γδ T cells contained more cytotoxic granules than their counterparts in the liver.

Discussion Collectively, our preliminary data suggest that CD8 T cells and innate immune cell subsets are likely to mediate liver damage and fibrosis progression in NASH patients. Especially the role of the innate T cell subsets warrants further exploration towards their specific role in progression from NAFLD to NASH.

3.22 Europäische Studie: Heterozygoter Alpha-1-Antitrypsinmangel (Pi*MZ) führt zu einem intermediären Leber-Phänotyp

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Hintergrund Die Pi*Z-Mutation im SERPINA1 Gen führt zur Akkumulation von abnormalem Alpha-1-Antitrypsin (AAT) in Hepatozyten. Dies führt zu Einschlusskörpern und proteotoxischem Stress. Die heterozygote Variante (Pi*MZ) stellt einen wichtigen Risikofaktor für die Entwicklung einer Leberzirrhose bei Individuen mit einer vorbestehenden Lebererkrankung dar. Um ihren Stellenwert in der Gesamtpopulation zu klären, haben wir Pi*MZ-Individuen mit Trägern einer homozygoten Pi*Z-Mutation (Pi*ZZ), jeweils ohne vorbestehende Lebererkrankung, verglichen.

Methode Für die Studie haben wir über unser europäisches, multizentrisches, Register 407 Pi*ZZ Patienten, 295 Pi*MZ Individuen und 235 Kontrollprobanden ohne AAT (Genotyp: Pi*MM) rekrutiert. Die Patienten wurden klinisch und durch Erhebung von Laborwerten standardisiert erfasst. Durch transiente Elastografie wurden die Lebersteifigkeit (LSM, Fibrosemarker) und controlled attenuation parameter (CAP, Marker für Steatose) gemessen. Andere Komorbiditäten der Leber wurden ausgeschlossen. Bei 33 der Patienten mit Pi*MZ und 35 Pi*ZZ- Probanden wurde eine Leberbiopsie durchgeführt. Das Gewebe wurde in HE, PAS-D und immunhistochemisch (Pi*Z-spezifische Antikörper) gefärbt. Der Inhalt der Einschlusskörper wurde quantifiziert.

Ergebnisse Pi*MZ Träger wiesen höhere AST, GGT und LSM (p<.05)-Werte als Pi*MM-Probanden auf. 8% der Pi*MZ Patienten hatten LSM ≥7,1 kPa als Hinweis auf eine signifikante Fibrose (vs. 1% von Pi*MM; p<.001). Pi*MZ Individuen hatten geringere AST, GGT, LSM und CAP Werte als Pi*ZZ-Individuen (p<.05). Im Gegensatz zu Pi*ZZ-Probanden, haben die Pi*MZ Teilnehmer weder erhöhte Lebersteatose noch metabolische Alterationen (p<.0001).

Tabelle 1 Histologische Untersuchungen von Pi*MZ und Pi*ZZ Individuen. Die Fibrosegrade werden nach Kleiner klassifiziert; die Anzahl der Einschlusskörper wird von 0 (keine) bis 4 (viele) eingestuft.

Routinefärbungen (HE+PAS-D) zeigten Einschlusskörper in <20% der Pi*MZ Träger (A, B). In der Immunhistochemie wurden bei <40% der Pi*MZ Patienten Einschlüsse festgestellt (C). Je höher das Stadium der Fibrose bei Pi*ZZ-(E) und Pi*MZ-Probanden (F), desto mehr Einschlusskörper gab es.

Fazit Im Vergleich zu Pi*MM und Pi*ZZ Patienten, weisen Pi*MZ Träger einen intermediären Phänotyp auf. Fortgeschrittene Leberfibrose ist charakterisiert durch eine erhöhte Anzahl an Einschlusskörpern.

3.23 Der angiokrine HGF-Signalweg steuert die Organ- und Körpergröße sowie die Hepatozytenproliferation

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Einleitung Hochspezialisierte Leber-Sinus-Endothelzellen (LSEC) steuern Organfunktion, Stoffwechsel und Entwicklung der Leber durch die Sekretion sogenannter Angiokine. LSEC exprimieren den Hepatozytenwachstumsfaktor (HGF), der an der pränatalen Entwicklung, der metabolischen Homöostase und der Leberregeneration beteiligt ist. Der genaue Einfluss des von LSEC stammenden HGFs zu dessen verschiedenen Funktionen ist bisher weitestge-

hend unerforscht. Ziel der Arbeit war die Untersuchung der hepatischen angiokrinen HGF-Signalgebung während der physiologischen Homöostase und der Leberregeneration.

Methodik Stab2-iCretg/wt;HGFfl/fl (HGFΔLSEC)-Mäuse wurden generiert, um die HGF-Expression in LSEC von der frühen Embryonalentwicklung an selektiv zu deletieren. In diesem Zusammenhang wurde die globale Entwicklung, die metabolische und endotheliale Zonierung sowie weitere Organfunktionen bewertet. Um die Leberregeneration zu untersuchen, wurde erstmalig eine 70% ige partielle Hepatektomie (PH) an diesem Mausstamm durchgeführt und die Kinetik der Regeneration, der Hepatozytenproliferation und der HGF/c-MET-Signalwege näher analysiert.

Ergebnis HGFΔLSEC -Mäuse waren lebensfähig und fruchtbar. Obwohl die metabolische und endotheliale Zonierung sowie das Verhältnis von Leber zu Körpergewicht nicht verändert waren, konnte bei HGFΔLSEC-Tieren ein reduziertes Gesamtkörper- und Gesamtlebergewicht nachgewiesen werden. Das Auftreten nekrotischer Areale in den Lebern war bei HGFΔLSEC M-Mäusen stärker ausgeprägt und die Regeneration 72 Stunden nach PH verzögert. Diese Beobachtung war mit einer verringerten Hepatozytenproliferation 48 Stunden nach PH verbunden. Der HGF/c-MET-Signalweg war bei HGFΔLSEC weniger aktiv und die Aktivierung dieser Achse beeinträchtigte die Herabregulierung des anti-apoptotischen Proteins Deptor, was in diesem Zusammenhang ein neues Ziel dieses Signalwegs darstellt.

Schlussfolgerung Angiokrines HGF ist an der Kontrolle des Körper- und Organwachstums sowie an den frühen Stadien der Leberregeneration nach PH beteiligt, um übermäßige Organschäden zu verhindern.

3.24 Abcb4-knockout reduces hepatic lipid steatosis in HBs transgenic mice

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Introduction ATP-binding cassette subfamily B member 4 (ABCB4) is a phospholipid translocator present at the canalicular membrane of hepatocytes, which "flops" phosphatidylcholine into bile. Abcb4-/- mice represent the most reproducible *in vivo* model to study chronic cholestatic liver diseases. Abcb4-/- mice exhibit a dysregulated lipid metabolism which is associated with disease pathogenesis. Hepatitis B transgenic mice (HBs) spontaneously develop hepatic steatosis. We previously reported that Hepatitis B virus surface proteins accelerate cholestatic injury and tumor progression in HBs/Abcb4-/- double-transgenic mice. With the current study, we aimed to investigate the effect of Abcb4-/- induced cholestasis on the hepatic lipid metabolism in HBs transgenic mice.

Methods Hybrids of HBs transgenic mice and Abcb4-/- mice were bred on BALB/c background. qPCR, Western blot, Oil Red O staining, High-performance thin-layer chromatography (HPTLC) and immunohistochemistry (IHC) methods were used to characterize alterations in lipid metabolism.

Results Hepatic neutral lipid depots were found in HBs transgenic mice. In comparison, intracellular lipid storage was remarkably reduced in the hepatocytes of both, Abcb4-/- and HBs/Abcb4-/-. Similarly, HPTLC based lipid quantification of the liver tissues revealed a significant reduction in the amount of triacylglycerol (TAGs) while the amount of free fatty acids was significantly increased in Abcb4-/- and HBs/Abcb4-/- in comparison to wild type and HBs mice, respectively. Serum analysis also showed a reduction in TAGs and cholesterol levels. The expression level of perilipin2 (PLIN2), a lipid droplet associated protein, was reduced in Abcb4-/- and HBs/Abcb4-/-. Reduction of PLIN2 was associated with suppression of TAGs synthesis and *de novo* lipogenesis associated gene expression (MGAT1, DGAT1, FASN, HMG-CoA, ACC1, and SREBP1-c, PPARγ). We further analysed regulatory pathways that can regulate intracellular lipid metabolism and PLIN2, found that PLIN2 can be regulated through the activation of AMPK and CREB signaling pathways.

Conclusion Cholestatic conditions mediated by the Abcb4 knockout reduced lipogenesis in the hepatocytes of HBs transgenic mice, thus ameliorating intrahepatic steatosis.

3.25 *Apoe*- vs. *Ldlr*-KO: The genetic background determines the function of Oncostatin M in the regulation of lipid homeostasis

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Background Oncostatin M (OSM) is a member of the interleukin-6-type cytokine family, which plays a pivotal role not only in inflammatory processes including the acute phase response, but also in the regulation of metabolic processes. Although pathogenetically not entirely elucidated, growing evidence emphasizes the importance of OSM in the highly prevalent metabolic syndrome. Previous studies revealed increased body weight, hepatic steatosis and insulin resistance in OSM receptor beta-deficient aged mice on chow diet. On high-fat diet, these effects were even more pronounced.

Question To investigate the metabolic features of Oncostatin M in two different mouse models prone to atherosclerosis and liver steatosis.

Methods We employed *Apoe*-/- and *Ldlr*-/- single knockout as well as *Ldlr*-/-*Osmr*-/- and *Apoe*-/-*Osmr*-/- double knockout mice. Mice were fed a Western-type diet for 12 weeks. Thereafter, mice were sacrificed and serum lipid levels were measured by using enzymatic assays and lipoprotein fractions were determined by performing HPLC analyses. Hepatic lipid content was studied following Folch's lipid extraction protocol. Gene expression analyses of adipose and liver tissue were carried out using the RT-qPCR.

Results Interestingly, *Ldlr*-/- single knockout mice exhibited the most pronounced weight gain during the Western-type diet, fitting to increased expression levels of the very-low-densitiy-lipoprotein receptor in adipose tissue. Lipid assays revealed a contrasting impact of *Osmr* deficiency on serum levels of cholesterol, non-esterified free fatty acids and triglycerides in the background of *Apoe*-/- and *Ldlr*-/- mice. Intriguingly, rather total cholesterol levels than serum lipoprotein fractions appeared to be affected since VLDL/LDL/HDL distribution differed just slightly within each group and its respective *Osmr*-/- correlate. Besides, similar trends in altered cholesterol and triglyceride homeostasis were found in the livers of our mice. Differently regulated inflammatory markers of liver and adipose tissue imply a complex interplay between lipid metabolism and inflammation.

Conclusion Depending on the genetic background, OSM appears to be protective or pathogenic in the development dyslipidemia. Our experiments indicate that the level of inflammation plays a crucial role in this decision. Further in depth characterization of the mouse models is required to understand the underlying molecular mechanisms.

3.26 Phenotyping non-alcoholic fatty liver disease by the gut microbiota – ready for prime time?

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Background and aims Several studies suggest an association of a specific gut microbiota signature with the presence of non-alcoholic fatty liver disease (NAFLD). However, analyzed patient populations and methods strongly differ among these studies. Therefore, the aim of this study is to examine the reproducibility of previous human data on NAFLD in our patient cohort using next generation sequencing (NGS) methods.

Patients and methods The individual taxonomic intestinal microbiota composition of 111 subjects was analyzed using 16S rRNA gene sequencing. Studies investigating the gut microbiota in NAFLD patients were identified from PubMed listed publications and the results were summarized. The study participants were grouped according to their disease stage and the microbiota composition was compared to findings of the identified studies.

Results Results from 13 identified studies were compared to our data. Our cohort included 90 NAFLD patients (n = 20 NAFL (non-alcoholic fatty liver); n = 47 NASH (non-alcoholic steatohepatitis); n = 23 without liver biopsy) and 21 healthy controls. A decreased abundance of the phylum Bacteroidetes and the *Ruminococcaceae* family as well as an increased abundance of the *Lactobacillaceae* and *Veillonellaceae* family and *Dorea* genus were the most frequently reported changes among NAFLD patients in 4/13, 5/13, 4/13, 2/13, 3/13 studies, respectively. Whereas these alterations in gut microbiota composition were also observed in our patient cohort, the majority of published differences could not be reproduced, neither in our own nor in other NAFLD cohort studies.

Conclusions The most frequently reported dysbiotic changes of NAFLD patients, namely decreased abundances of Bacteroidetes and *Ruminococcaceae* could also be identified in our patient cohort. Despite repeatedly reproduced abundance patterns of specific bacteria, the heterogeneous study results do not allow to reliably phenotype NAFLD by gut microbiota up to now. Further prospective studies with homogenous patient cohorts and standardised methods are necessary to identify a consistent and disease-specific gut microbiota signature in NAFLD patients.

3.27 Human 3-dimensional liver organoids: in vitro model systems to study liver diseases

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Introduction There is by now a common agreement that 2-dimensional, monolayer cell cultures, even if they are composed of primary isolated cells, do not sufficiently reflect the in vivo situation. Therefore emerging effort is created to generate 3-dimensional tissue culture models, so called organoids. Accordingly, the goal of the present study is to generate organoids from quasi primary human liver cells (upcyte® cells; standardizable model) as well as from cells isolated from resected human liver tissue (personalized model).

Methods Human upcyte® hepatocytes and LSEC (liver sinusoidal endothelial cells) were combined with primary isolated human HSC (hepatic stellate cells) in a physiological ratio of 70:25:5, respectively. In parallel human liver tissue from the surrounding of HCC was dissolved using different mixtures of enzymes. For both setups, the cells were then cultured inside of matrigel droplets using diverse recipes of culture medium. Organoids were characterized by real-time PCR for diverse cell type and functionality markers as well as H&E- and immunofluorescent stainings.

Results Upcyte® organoids initially formed well in a medium containing stem cell/niche factors. They were then propagated in a more basal medium and were used to study e. g. hepatic responses to ethanol. This setup was nicely reproducible and resulted in epithelial (hepatocyte-containing) organoids, surrounded and connected by non-parenchymal cell structures (HSC and LSEC). When organoids were grown from primary isolated liver cells, they also needed the presence of specific stem cell factors in the medium. So far we did not manage to keep the primary non-parenchymal cells from the resected specimens alive so that the resulting organoids contained only hepatocytes. In contrast to the upcyte® organoids, the primary tissue-derived ones kept growing and could be propagated at least until passage 5.

Summary and conclusions We successfully generated human liver organoids that can in the future be used to study liver diseases and for drug toxicity approaches. Upon further optimization of the protocol the upcyte® organoids will especially be useful for basic science research whereas those from primary patient tissue will finally be tested for personalized medicine applications.

3.28 Characterisation of enzyme activities in liver parencyhma of diabetic ChREBP-KO mice

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Question Intraportal pancreatic islet transplantation (IPIT) results in local hyperinsulinism and hyperglycemia in streptozotocin-induced diabetic mice. Metabolic changes emerge in hepatocytes downstream of transplanted islets including an activation of glycolysis and lipogenesis corresponding to glycogen storing clear-cell foci of altered hepatocytes (CCFs). Furthermore, murine CCFs are preneoplastic lesions and progress to hepatocellular adenomas and hepatocellular carcinomas (HCC). To analyse carbohydrate responsive element binding proteins (ChREBP) role in regulating glycolysis, lipogenesis and gluconeogenesis regarding the activities of main metabolic enzymes such as glucose-6-phosphatase (G-6-Pase; gluconeogenesis), glucose-6-phosphate dehydrogenase (G-6-PDH; pentose phosphate pathway) and glucokinase (GK; glycolysis) the transcription factor was knocked out in mice and combined with the IPIT model for hormonally induced hepatocarcinogenesis.

Methods Frozen liver tissue specimen of wild type (WT) and ChREBP-KO (KO) mice containing CCFs, HCCs and unaltered liver tissue was cut as serial sections of 10 and 14 µm thickness, mounted on a slide, stained (H&E, PAS, enzyme histochemistry: lead method (G-6-Pase), improved PVA and tetrazolium salt NBT method (G-6-PDH and GK)) and then semiquantitatively compared (18 groups consisting of WT/ChREBP-KO; streptozotocine-induced diabetic/non-diabetic; with/without IPIT; 1, 4, 24 and 48 weeks).

Results In non-diabetic WT mice, we observed a regular storage of glycogen from 1 until 48 weeks. Activities of G-6-Pase and G-6-PDH were strongest in acinar zone 1 and faded to zone 3. In contrast, the activity of GK was pronounced in acinar zone 3. Diabetic WT mice revealed a slightly decreased glycogen storage, a strongly increased G-6-Pase and both G-6-PDH and GK were decreased. These patterns are in line with previous observations in rat liver tissue specimen.

In both non-diabetic and diabetic KO mice glycogen storage was higher, activity of G-6-Pase strongly whereas activities of G-6-PDH and GK were only

slightly decreased. Moreover, preneoplastic CCFs and HCCs revealed a strong upregulation of GK in WT and KO mice.

Conclusion Murine CCFs resemble preneoplastic liver lesions in the rat in terms of carbohydrate related enzyme activity patterns. The altered activities of G-6-Pase, G-6-PDH and GK support the idea of ChREBP as a part in the switch from a glycogenotic to a lipogenic phenotype in preneoplastic lesions.

3.29 Suppression of bile acid-CoA:amino acid N-acyltransferase gene expression by Oncostatin M

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Question Chronic inflammatory diseases have been associated with an altered serum bile acid composition which by acting on farnesoid X receptor or G-protein-coupled bile acid receptor 1 (GPBAR1) might act as signaling molecules to affect disease progression. While primary bile acid synthesis and conjugation take place in the liver, secondary bile acids are generated by microbiota in the gut. Therefore, diseases affecting liver metabolism or microbiota composition, like non-alcoholic fatty liver disease, might directly alter the expression of enzymes involved in bile acid synthesis and/or conjugation. Here, we investigated the influence of interleukin-6 (IL-6) and oncostatin M (OSM) on gene expression of bile acid-CoA:amino acid N-acyltransferase (BAAT) which catalyzes the taurine/glycine-conjugation of primary bile acids in the liver.

Methods Immortalized human hepatocytes (IHH) and human hepatoma cells (HepG2) were stimulated with IL-6 or OSM in different concentrations and for different periods of time. Quantitative real-time PCR was performed to evaluate gene expression. Pharmacological inhibitors were used to address the signaling pathways involved in gene regulation.

Results We found a pronounced decrease in the expression of *BAAT* in response to OSM, while stimulation with IL-6 had a much weaker effect. The OSM-mediated suppression of *BAAT* expression persisted over at least 24 h even after wash-out of the cytokine. This long-lasting effect of OSM most likely relies on the strong adhesion of bio-active OSM to the extracellular matrix, in particular to collagen. Using pharmacological inhibitors, we could show that abrogation of the Janus kinase activity completely blocks the suppressive effect of OSM, while inhibition of the stress-activated MAP kinases p38 and JNK had no effect. Inhibition of ERK1/2 activation partially restored gene expression of *BAAT* indicating a contributing effect of these MAPK to the suppressive activity of OSM.

Conclusions We could identify a so far unrecognized suppressive activity of the interleukin-6-type cytokine OSM on the gene expression of the enzyme catalyzing the final step in taurine/glycine-conjugation of bile acids in the liver, *BAAT*. This could indicate an involvement of OSM in altered bile acid conjugation resulting in potential increased liver injury upon intrahepatic OSM release.

3.30 *Schistosoma mansoni* eggs modulate lipid metabolism in hepatocytes

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Introduction Schistosomiasis is a prevalent parasitic disease causing severe clinical symptoms as well as socioeconomic problems and more than 200,000 deaths per year. During infection with *S. mansoni*, paired adult worms produce approximately 300 eggs per day per couple. Eggs-secreted and immunologically active antigens are involved in the recruitment of inflammatory and immune cells, leading to the formation of granulomas and eventually progressing fibrosis.

Methods In the present study, liver tissue of *S. mansoni*-infected hamsters was examined by Western blot (WB) and immunohistochemistry (IHC) to analyse hepatic lipid metabolism during *S. mansoni* infection. Monosex infection with cercariae of one sex was used as control to mimic worm infection without egg production.

Results IHC analyses showed increased Perilipin2, FAS (fatty acid synthase), and ACC 1 (Acetyl-CoA carboxylase 1) expression in hepatocytes around granulomas in bisex infected livers. Additionally, WB demonstrated increased FAS and ACC 1 expression in the livers of bisex-infected hamsters as compared to monosex. On the other hand, enzymes involved in lipid catabolism, e. g. hepatic lipase (LIPC), were found to be downregulated in hepatocytes around granulomas.

Conclusions In conclusion, our data demonstrated for the first time enhanced lipid synthesis and accumulation as well as reduced lipid catabolism in hepatocytes around granulomas in infected liver tissue. These results suggest that eggs might not only benefit from the host"s lipids as they cannot produce these lipids by themselves but, moreover, that eggs are able to actively drive the process of lipid synthesis and supply. Since it is not clear if this processes are controlled directly by egg-secreted factors or indirectly by the induction of granulomatous inflammation we aim to analyse whether these effects can be induced by eggs or egg-secreted factors in cell culture.

3.31 Augmenter of Liver Regeneration (ALR) reduziert die Gallensäure induzierte Apoptose

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Gallensäuren (GS) besitzen amphiphile Eigenschaften und sind essentiell für die Absorption von Nahrungsfetten, Vitaminen und anderen Nährstoffen. Weiterhin können GS auch Entzündungsreaktionen und Apoptose induzieren. Die Synthese der GS findet in den Hepatozyten statt und wird in erster Linie durch das Schlüsselenzym CYP7A1 (Cholesterol 7α hydroxylase) gesteuert. Die Expression von CYP7A1 wird von Transkriptionsfaktoren reguliert und ist während des Regenerationssprozesses der Leber (partielle Hepatektomie) reprimiert, damit eine Akkumulation von toxischen GS in der verbleibenden Hepatozyten vermieden wird. Bei Leberschädigung werden mitogene Faktoren freigesetzt u. a. ALR (Augmenter of liver regeneration), ein hepatotrophes Protein mit anit-oxidativen und anti-apoptotischen Eigenschaften. Über eine Rolle von ALR bei der Regulation von CYP7A1 und einem potentiell schützenden Einfluss auf Heptozyten durch Verringerung GS induzierter Apoptose ist bisher noch wenig bekannt.

In einem *in vitro* Modell wurden humane Hepatozyten (Hepatoma Zellinien, primäre humane Hepatozyten) mit der toxischen GS Glycochenodeoxycholic acid (GCDCA, 100 µM) und rekombinant humanem ALR (rhALR, 100 ng/ml) inkubiert und die Expression on CYP7A1 sowie GS induzierte Apoptose analysiert. Wir konnten zeigen, dass ALR Behandlung zu reduzierten GS-Spiegeln im Kulturmedium führt und dies auf eine Reduktion von CAR (Constitutive Androstone Receptor) und in der Folge von CYP7A1 Expression zurückzuführen ist. Weiterhin konnte ALR eine durch GCDCA induzierte Apoptose (intrinsische Apoptose) verringern, was jedoch nicht für die Expression von DR5 (death receptor 5) oder die TRAIL induzierte Apoptose (extrinsische Apoptose) gezeigt werden konnte (Caspase 3/7 assay, bax western blot). Applikation von rhALR induziert den PI3/Akt Signalweg (Aktivierung von Akt) und verstärkt die Phosphorylierung von GSK3b, was in der Folge zur Reduktion von bax Expression und Apoptose führt.

Zusammenfassend, ALR schützt Hepatozyten vor Akkumulation toxischer GS und GS induzierter Apoptose und weist daher auf eine neue Rolle von ALR während der Leberregernation hin.

3.32 The interaction of Hedgehog and mTor signaling in healthy hepatocytes

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Introduction The Hedgehog (Hh) pathway is one of the central morphogenetic pathways. It plays a key role in embryonic development and is a regulator of regeneration and stem cell function in adult tissues. Abnormal Hh Signaling contributes to different diseases like hepatic steatosis and cancer. Studies showed various interactions of the Hh pathway and the signaling of the mechanistic target of rapamycin (mTor) in different cancer types. The mTor pathway is an important mediator between nutritional signals and their appropriate cellular responses. However, the mechanisms behind the interaction of Hh and mTor Signaling are poorly understood, particularly in healthy tissue. In our studies, we demonstrate synergistic effects of both pathways in controlling healthy liver metabolism.

Methods Primary hepatocytes from C57Bl6/N mice were treated with the Hh inhibitor Cyclopamine, the mTorC1 inhibitor Rapamycin, the mTorC1/C2 inhibitor Torin and the combinations thereof. The cells were analyzed using Western Blots, qPCR and Seahorse technology.

Results Our experiments show synergistic inhibition by Cyclopamine and Rapamycin of p70S6 phosphorylation, a downstream kinase of mTorC1. Cyclopamine alone and Rapamycin alone have no or only a weak influence on the phosphorylation, respectively. When we repress mTorC1 and C2 with Torin, we see the same effect as with Cyclopamine and Rapamycin. However, the addition of Cyclopamine to Torin has no further influence on the phosphorylation state. In addition, we see this effect also in the phosphorylation state of Rictor, an mTorC2 component. Moreover, although the inhibition of the Hh pathway alone has no impact on the functionality of mitochondria, our Seahorse analyses show an increased inhibition of the electron transport chain in cells incubated with Rapamycin and Cyclopamine compared to Rapamycin alone.

Conclusion We suppose that the minor impact of Rapamycin on mTor pathway activity is an effect of the feedback mechanism via mTorC2. Therefore, our results suggest a possible interaction of the Hh pathway with mTorC2 signaling. We hypothesize that the Hh pathway may influence the energy metabolism and other cellular responses through the modulation of mTor signaling. This should be considered as potential side effects when using Hh pathway inhibitors as part of therapeutic intervention in cancerous diseases.

3.33 Hedgehog Signaling as a mediator between liver and adipose tissue

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Question In recent years it has become apparent that the morphogenic Hedgehog (Hh) pathway is not only active during embryonic development, but also in adult organs, controlling metabolism and maintaining homeostasis. The liver in particular as a metabolically active organ with many functions in energy homeostasis is of interest, especially since active Hh signaling could be detected in hepatocytes.

Crosstalk between the liver and tissues of the periphery such as the adipose tissue (AT) has long since been known. Prior experiments have revealed that inactivation of Hh in hepatocytes leads to morphogenic changes in several tissues, one of which is the AT. The mechanism and implications of such a Hh-dependent crosstalk between the tissues remain to be elucidated.

Methods Primary hepatocytes as well as the different types of AT – visceral (VAT), subcutaneous (SAT) and brown (BAT) – were isolated from two mouse strains with specific inactivation of Hh signaling in hepatocytes. Morphologic and biochemical characterization of AT by immunohistochemistry including analysis of adipocyte size as well as by molecular biological methods such as qPCR was performed.

Results Mice with an inactivation of Hh signaling in hepatocytes have a distinct phenotype in AT: an increase of all types of AT in both sexes as well as a changed distribution of cell size could be detected. Furthermore there are changes in the gene expression profile. Brown and beige adipocyte markers show increased expression, and lipid metabolism, in particular lipogenesis, seems to be affected in AT. Immunohistochemistry revealed UCP-1-positive cell clusters in VAT and SAT, indicating a browning effect.

Conclusions A hepatocyte-specific inactivation of Hh leads to phenotypic and metabolic changes in the AT of mice, resulting in the emergence of brown adipocytes in SAT and, more intensely, in VAT. Although the mechanism of Hh-mediated crosstalk between liver and AT remains elusive, the results reveal a novel and highly interesting aspect of how morphogenic pathways control metabolism through inter-organ communication.

3.34 Glucose and metformin differentially regulate FGF19 signaling in human HepG2 cells

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Background Fibroblast growth factor 19 (FGF19) is a physiological key regulator of various metabolic processes such as hepatic bile acid synthesis as well as carbohydrate and lipid metabolism. On the other hand, pathological hyper-activation of FGF19 expression and down-stream signaling via fibroblast growth factor receptor 4 (FGFR4) has been observed in various human cancers including hepatocellular carcinoma. In this ongoing project, we aim to analyze the molecular mechanisms that regulate the FGF19/FGFR4 pathway in the liver under physiological and pathological conditions.

Methods Human HepG2 cells were cultivated under conditions of glucose abundance or glucose deprivation (high or low glucose medium) and stimulated with recombinant FGF19. Activation of the FGF19/FGFR4 signaling pathway was quantified via Western blot and qPCR. The anti-diabetic drug metformin as well as the AMP analog AICAR were used to test a potential contribution of the AMP-activated protein kinase (AMPK) to the regulation of FGF19/FGFR4 signaling. Cell proliferation was monitored using the MTT assay.

Results Only cultivation of cells with high glucose medium allowed for robust activation of FGF19 signaling as indicated by increased phosphorylation of ERK1/2 and strong regulation of FGF19 target genes. In contrast, HepG2 grown in low glucose medium showed much weaker activation of pERK1/2 and less pronounced regulation of target genes after FGF19 stimulation.

Vice versa, pre-treatment with the anti-diabetic drug metformin or the AMP analog AICAR blunted FGF19 signaling in cells cultivated in high glucose medium. Moreover, metformin pre-treatment also inhibited the proliferation of HepG2 cells in the presence of FGF19. Interestingly, inhibitory effects of metformin and AICAR on FGF19 signaling were also found in a human non-cancer hepatocyte cell line (i.e. immortalized human hepatocytes, IHH).

Conclusion This study suggests that the availability of cellular energy substrates such as glucose has a significant impact on the FGF19/FGFR4 signaling pathway in liver cells. Moreover, our data point to a potential involvement of the AMP-activated protein kinase (AMPK) in the regulation of this process and indicate that the anti-diabetic drug metformin may be useful for pharmacological inhibition of hyper-activated FGF19 signaling under pathological conditions. Further analysis using in vivo models is therefore warranted.

3.35 Investigation of the glycogen-associated proteome via proximity-biotinylation

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Background Glycogen metabolism is upregulated in many tumor types, such as hepatocellular carcinoma (HCC) of the clear cell phenotype. These cells are massively loaded with glycogen, resulting in the name-giving clear cell phenotype in HE-stained tissue. The massive storage of glycogen suggests that this may be an important aspect of the pathophysiology of cancer cells, since glucose can be provided for the aerobic glycolysis and the anabolic metabolism can be sustained via the pentose phosphate pathway. However, the impact of glycogen masses on protein distribution within the cell is not clear. Consequences could include the enrichment or depletion of certain proteins or organelles.

Aim For this purpose, the glycogen-associated proteome should be determined in glycogen-overloaded HepG2 cells (an immortal cell line consisting of human liver carcinoma cells).

Methods Therefore, we established and optimized a TurboID-system, based on the principle of proximity-biotinylation.

Results Overexpression of hyperactive glycogen synthase 2 (ha-GYS2) resulted in massive glycogen loading, evident in PAS-stained HepG2-cells and electron microscopy. After fusion of TurboID to ha-GYS2 glycogen overload failed, but the co-transfection with ha-GYS2 at a molar ratio of 1:5 – 1:6 could restore glycogen overload. By western blot analysis we confirmed that enough ha-GYS2-TurboID was expressed to obtain sufficient biotinylation. We also could prove co-localization of ha-GYS2/ha-GYS2-TurboID, indicating that ha-GYS2-TurboID is not mislocalized. Fluorescence microscopy with fluorescent streptavidin in combination with PAS-staining showed that in approximately 75% of cells containing biotinylated proteins also glycogen had accumulated. In further investigations could indicate that the glycogen was soluble and metabolically accessible: It could be depleted by culture in glucose-free medium or digestion with alpha-amylase before PAS staining and biotin-labeled proteins were detected within glycogen masses.

In summary, our results show that labeled proteins co-localize with ha-GYS2 but also biotinylation is diffusely associated with glycogen. Therefore, we are able to label proteins in the proximity of glycogen, giving us the opportunity to purify biotinylated proteins and determine the glycogen-associated proteome in further investigations.

3.36 *E. coli* bacteria trigger mucin reduction to promote a destabilized epithelial barrier in SBP

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Background Spontaneous bacterial peritonitis is a life-threatening complications of liver cirrhosis, that is driven by bacterial translocation. Bacterial translocation means that commensal gut bacteria translocate paracellular *via* dysregulated cell-to-cell contact proteins to mesenteric lymph nodes. Although a disrupted intestinal epithelial barrier of patients with liver cirrhosis and SBP have already been shown by us, we and others do not know the detailed mechanisms of SBP development. Therefore, we established a clinically relevant Caco-2 model to identify molecular mechanisms of bacterial translocation.

Methods Caco-2 cells were cultivated for six days and treated with different concentrations of *E. coli* bacteria that were isolated from ascitic fluid of two patients with liver cirrhosis and SBP. As control, heat-inactivated bacteria (65 °C, 5 min) and *E. coli* LPS O55:H5 (0,1 and 1 µg/ml) were used for stimulation of Caco-2 cells. For the study of mechanisms of paracellular bacterial translocation, cell-to-cell contact proteins (E-cadherin and occludin) were examined on RNA and protein level. Caco-2 cells were stimulated with *E. coli* bacteria directly or *via* a semipermeable membrane. In addition, mucin regulation (MUC-2 and MUC5AC) of Caco-2 cells were analyzed on RNA level after cells were incubated with SBP *E. coli* bacteria.

Results Bacterial stimulation with *E. coli,* isolated of patients with liver cirrhosis and SBP, resulted in a decrease of E-cadherin and occludin of Caco-2 cells. The reduction intensity was dependent on the *E. coli* strain that was used for stimulation. Heat-inactivated bacteria and LPS failed to induce any changes. If Caco-2 cells were incubated with *E. coli* bacteria directly, a greater downregulation, especially for E-cadherin was shown. Moreover, SBP *E.* coli bacteria reduced mucin production of Caco-2 cells.

Conclusion For a paracellular translocation, *E. coli* bacteria destabilize intestinal epithelial barrier *via* dysregulated cell-to-cell contact proteins. Direct interaction of *E. coli* bacteria and intestinal epithelial cells promote downregulation of cell-to-cell contact proteins. Therefore, SBP *E. coli* bacteria trigger downregulation of mucin regulation to facilitate a physical interaction of bacteria and the intestinal epithelium.

3.37 Antioxidant-based therapy in non-alcoholic steatohepatitis (NASH) and NASH-induced hepatocellular carcinoma development

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Life-style alterations such as high caloric intake combined with a sedentary lifestyle have highly augmented over the last decades the number of obese people in Western Countries, and it's expected to increase further in the near future. Non-alcoholic fatty liver disease (NAFLD) represents the most common liver morbidity, characterized by excessive fat accumulation (steatosis). Around 30% of patients having NAFLD, develop non-alcoholic steatohepatitis (NASH) that shows beyond steatosis, also chronic inflammation, hepatocellular damage and fibrosis. The pathogenic condition of NASH constitutes a major risk factor for hepatocellular carcinoma (HCC), the most common primary liver malignancy and the third most common cause worldwide of cancer-related death. To date, the exact mechanism underlying NASH and NASH-induced HCC is still unknown. In the context of NASH, high caloric diet leads to oxidative stress originated by the increased levels of radical oxygen species (ROS) produced mainly by endogenous aberrant mitochondria, endoplasmic reticulum (ER) and peroxisomes. The final effect of overproduction of ROS is the enhancement of lipid peroxidation and protein oxidation, thus leading to a detrimental effect in the homeostasis of fatty acids in the liver and to ER stress. Especially the lipid peroxidation is considered a relevant source of mutagens triggered by ROS. Persistent oxidative stress modifies lipids, proteins and DNA; while it also leads to a deregulated immune response and increased proliferation, eventually favoring tumorigenesis. This is relevant not only in livers of NASH affected patients but also in HCC cases. To study the role of ROS in NASH and in NASH-induced HCC mouse models, we tested in combination with choline-deficient high-fat diet (CDHFD), a xanthophyll carotenoid compound. Our aim was to assess whether therapeutic treatment with an antioxidant in the presence of NASH CDHFD-induced, would ameliorate the NASH phenotype and reduce cancer incidence. The prophylactic treatment led to a lower NAFLD activity score. In addition, it decreased significantly hepatic inflammation and proliferation. The therapeutic treatment instead, reduced the cancer incidence. Our data indicate that oxidative stress is indeed an important driver of tumorigenesis that should be considered in NASH therapeutic practice in order to reduce cancer risk.

3.38 New role of copper transporter ATP7B in lipid metabolism of enterocytes

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Mutation of the copper exporter ATP7B is the cause of Wilson's disease and is associated with toxic accumulation of copper (Cu) in different tissues. The role of ATP7B in the intestine is far from being understood.

Our aim is the analysis of an intestinal ATP7B KO cell line and the characterization of Cu/iron (Fe) and lipid metabolism.

The CRISPR/Cas9 system was applied to human intestinal CaCo-2 cells. Single cell cloning as well as bacterial cloning were performed for sequencing analysis. Toxicity was determined by MTT assay and determination of Cu by AAS analysis. Gene expression analysis (RT-qPCR) was performed. Lipid metabolism was assessed by EM, RT-qPCR, ELISA, triglyceride quantification and staining.

About 50% of the CaCo-2 cell clones subjected to CRISPR/Cas9 mediated ATP7B KO showed increased sensitivity to Cu. At 0.15 – 0.75 mM Cu, the sensitivity in the KO cells was significantly elevated. Knockin confirmed the role of ATP7B, whereas Fe showed no impairment of cell survival. DCYTB metal reductase (-4.9 ± 1), oxidative stress regulating HMOX1 (+14.8 ± 5) and metallothionein 1 (+56.2 ± 16) were significantly affected. A significant downregulation of the chylomicron structural protein ApoB48 (-3.5 ± 0.4) and APOE were observed in KO cells. Intracellular triglyceride levels decreased after Cu treatment.

The characterization of a novel intestinal ATP7B knockout cell line showed impairment of Cu homeostasis and lipid metabolism. The results extend our understanding of the lipid and Cu transport in enterocytes.

3.39 Effect of HE-relevant factors on GLAST clustering in cultured rat astrocytes

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Background Hepatic encephalopathy (HE) is a neuropsychiatric syndrome frequently accompanying liver failure. Currently, HE is seen as a clinical manifestation of a low grade cerebral edema and cerebral oxidative stress. HE is triggered by a heterogeneous set of factors which action integrates at the level of inducing osmotic and oxidative stress in astrocytes. Several studies have shown that glutamatergic neurotransmission in the brain is disturbed in HE. Glutamate is the main excitatory neurotransmitter in the brain and thus the extracellular glutamate concentrations are tightly controlled by two sodium dependent glutamate transporters: Glutamate transporter 1 (GLT-1) and glutamate aspartate transporter (GLAST). Recent studies showed that ammonia as well as astrocyte swelling per se induce the rapid release of glutamate by a calcium-dependent vesicular release mechanism in astrocytes. Long term treatment of cultured astrocytes with ammonia (>= 48 h) impairs glutamate uptake through downregulation of GLAST which may be a consequence of GLAST mRNA oxidation. Furthermore short term incubation with ammonia also impairs glutamate uptake in astrocytes in a sodium and ph-dependent way.

Aims and Methods Our study focuses on the rapid effects of ammonia on the intracellular GLAST localisation. Therefore we transfected cultured rat astrocytes with a vector coding for a YFP-tagged GLAST.

Results By using conventional epifluorescence microscopy an almost instantaneous clustering of GLAST-YFP molecules after exposure to NH4Cl (5 mmol/l) was detected. Also lower concentrations of NH4Cl down to 0,5 mmol/l were able to induce the clustering. A similar effect was seen after exposure to glutamate (100 µmol/l). CH3NH3Cl which causes a similar pH-change as NH4Cl did not lead to a clustering of the YFP-tagged GLAST molecules in cultured rat astrocytes. Further examinations showed that the clustering of GLAST is Ca2+ and NO dependant as it can be prevented by using the Ca-chelator BAPTA (10 µmol/l) or the inhibitor of NO-Synthase L-NAME (1 mmol/l) or induced by the NO-donor DEANONOate (50 µmol/l).

Conclusions and Outlook Our data suggest that in cultured rat astrocytes GLAST clusters rapidly after exposure to NH4Cl in an Ca2+ and NO dependent manner. In further studies we will closer analyse the lokalisation of the GLAST clustering using TIRF-microscopy and the possible multimerisation of GLAST using FRET-microscopy.

Lectures Session IV Tumors Saturday, February 15, 2020, 9:15 am – 10:00 am, Lecture Hall P1

4.1 Transcriptional profiling of tumor-specific CD8 T cells shows contribution of TIGIT to T cell exhaustion in liver cancer

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Hepatocellular carcinoma (HCC) is the second leading cause of cancer-related death and the fifth most common kind of cancer worldwide. This cancer has a 5-year survival rate of 10% and its increasing incidence requires the development of efficacious treatments against HCC. Recent advances in immunotherapy demonstrated its capability in treatment of cancer, but there is still potential for further development of immunotherapies against liver cancer. Immune responses against cancer are often hampered by upregulation of co-inhibitory receptors on the surface of CD8 T cells. This inhibition leads to emergence of T cell exhaustion, where tumor-infiltrating lymphocytes (TILs) show a reduced proliferative capacity and low production of effector cytokines IFNγ and TNFα, a mechanism that impedes tumor rejection by CD8 T cells. We have utilized the *Sleeping beauty*" (SB) and transposon system for the development of an autochtonous HCC mouse model. Using adoptive T cell transfer allowed us in-depth phenotyping of tumor-specific CD8 T cells and we could demonstrate pronounced upregulation of co-inhibitory receptors PD-1, TIM-3, CD160, LAG-3, 2B4 on T cell surface. The tumor-specific CD8 T cells also showed a reduced cytokine production and degranulation capacity, indicating the emergence of T cell exhaustion. In order to elucidate the molecular cause of tumor-induced T cell exhaustion we have performed the first whole transcriptome microarray analysis of tumor-specific CD8 T cells in a murine autochthonous liver cancer model, that allowed us to compare the mRNA profiles of naive, functional effector and exhausted tumor-specific CD8 T cells. The comprehensive transcriptomic data represents a means for the identification of candidate genes and pathways that play a role in T cell exhaustion. Particularly, the substantial upregulation of TIGIT suggested the involvement of this inhibitory T cell receptor in T cell exhaustion in liver cancer. Utilization of immune checkpoint-blockade against TIGIT in combination with PD-1 inhibition prolonged survival of tumor-bearing mice, compared to single inhibition of PD-1. We could further verify the expression of TIGIT on tumor-infiltrating CD8 T cells in patients with liver cancer. Our results suggest that TIGIT is involved in the appearance of T cell exhaustion in human liver cancer

and presents a potential target for combination treatment by immune checkpoint blockade.

4.2 Genomic characterization of cholangiocarcinoma in primary sclerosing cholangitis reveals novel therapeutic opportunities

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Background & Aims The lifetime risk of biliary tract cancer (BTC) in primary sclerosing cholangitis (PSC) patients exceeds 20% and BTC is currently the leading cause of death in PSC patients. To open new avenues for management of PSC-associated BTC (PSC-BTC), we aimed to delineate novel and clinically relevant genomic and pathological features of a large cohort of PSC-BTC tumor tissues.

Approach & Results We analyzed formalin fixed, paraffin embedded tumor tissue from 186 PSC-BTC patients from 11 centers in eight countries with all anatomical locations included. We performed tumor DNA sequencing at 42 clinically relevant genetic loci to detect mutations, translocations and copy number variations, along with histomorphological and immunohistochemical characterization. Irrespective of the anatomical localization, PSC-BTC exhibited a uniform molecular and histological characteristic similar to extrahepatic cholangiocarcinoma. We detected a high frequency of genomic alterations typical of extrahepatic cholangiocarcinoma, e.g. TP53 (35.5%), KRAS (28.0%), CDKN2A (14.5%), and SMAD4 (11.3%), as well as potentially druggable targets (e.g. HER2/ERBB2). We found a high frequency of non-typical/non-ductal histomorphological subtypes (55.2%) and of the usually rare BTC precursor lesion, intraductal papillary neoplasia (18.3%).

Conclusion Genomic alterations in PSC-BTC include a significant number of putative actionable therapeutic targets. Notably, PSC-BTC show a distinct extrahepatic morpho-molecular phenotype, independent of the anatomical location of the tumor. These findings advance our understanding of PSC-associated cholangiocarcinogenesis and provide strong incentives for clinical trials to test genome-based personalized treatment strategies in PSC-BTC.

4.3 NEMO prevents hepatocarcinogenesis independently of an IKK complex function by controlling liver regeneration

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The IKK complex is a central mediator of inflammatory NF-κB signaling – an important survival pathway in cancer cells. This complex consists of two catalytic subunits (IKKα and IKKβ) and a regulatory subunit (NEMO). Previous studies showed that the distinct IKK-subunits have functions beyond NF-κB signaling. The deletion of *Nemo* in parenchymal liver cells ($NEMO^{LPC\text{-}KO}$) induces apoptosis and compensatory proliferation leading to liver cancer. This specific phenotype does not occur by deleting the three NF-κB subunits (*RelA*/*RelB*/*c-Rel*). However, it is so far unknown if this presumed function of NEMO in hepatocarcinogenesis is associated with its role in controlling the I-κB-Kinase activity or if it is a completely independent function of NEMO in liver cells.

For this reason, we generated mice lacking all three IKK subunits in liver parenchymal cells ($IKK\alpha/\beta/NEMO^{LPC\text{-}KO}$). The phenotype of these triple knockout animals was compared with mice lacking both catalytic subunits ($IKK\alpha/\beta^{LPC\text{-}KO}$), enabling a functional dissection of the presumed I-κB-Kinase-independent function of the regulatory subunit NEMO. We show that the additional deletion of *Nemo* rescued $IKK\alpha/\beta^{LPC\text{-}KO}$ mice from lethal cholestasis and biliary ductopenia – a phenotype specifically observed in this double mutant mice. The loss of this specific IKK complex-independent function of NEMO further triggered apoptotic cell death of liver parenchymal cells (LPC) and induced a strong compensatory proliferation of the LPC compartment including cholangiocytes. In addition, the deletion of NEMO in $IKK\alpha/\beta^{LPC\text{-}KO}$ mice inhibited LPC necroptosis, which occurred in the double mutant mice. However, losing the expression of NEMO in this setting is not overall beneficial. We show that consistent triggering of hepatocyte death and compensatory proliferation leads again to spontaneous hepatocarcinogenesis.

Collectively, our data show that NEMO molecules unbound to the catalytic IKK subunits control LPC programmed cell death pathways and proliferation, and thereby cholestasis and hepatocarcinogenesis independently of an IKK-related function. Thus, NEMO and IKKα/β regulate LPC cell death and compensatory liver regeneration in functionally distinct ways. These results support the idea of different functional levels at which NEMO regulates I-κB-Kinase-independently liver inflammation and carcinogenesis.

Lectures Session V Viral Hepatitis and Immunology
Saturday, February 15, 2020, 11:45 am – 12:30 pm, Lecture Hall P1

5.1 Nanoparticle-mediated peptide delivery to liver sinusoidal endothelial cells protects from CD8 T cell-driven cholangitis

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Question We have previously shown that delivery of MHC II-restricted autoantigen peptides to liver sinusoidal endothelial cells (LSECs) using LSEC-targeting nanoparticles (NPs) provides effective protection from CD4 T cell-driven autoimmunity (Carambia et al. J Hepatol 2015). As LSECs are capable to cross-present exogenous peptides to CD8 T cells, we here investigated whether targeted delivery of MHC I-restricted autoantigen peptide to LSECs might serve antigen-specific treatment of CD8 T cell-driven autoimmunity. As a model, we used OT-I T cell-driven cholangitis in K14-OVAp mice expressing the cognate SIINFEKL peptide as autoantigen in cholangiocytes (Schwinge et al. J Immunol. 2015).

Methods LSEC-targeting NPs loaded with SIINFEKL peptide (SIINFEKL-NPs) or control NPs without peptide cargo were administered to K14-OVAp mice one day before transfer of pathogenic OT-I T cells. Five days after OT-I T cell transfer, clinical parameters of liver pathology were assessed and immunophenotyping of hepatic infiltrates, in particular of the transferred autoreactive CD8 T cells, was performed.

Results Adoptive transfer of OT-I CD8 T cells to K14-OVAp recipients induced liver inflammation and injury in control mice receiving unloaded NPs, while K14-OVAp mice receiving SIINFEKL-NP were largely protected from any signs of liver damage. Accordingly, upon SIINFEKL-NP treatment, serum transaminases were not elevated, and body condition remained unaffected, as compared to control animals, which showed elevated serum transaminase levels (ALT 33 vs. 140 U/mL; P = 0.0317; AST 159 vs. 617 U/mL) and poor body condition, including significant weight loss (weight change +0.86% vs. -8.95%; P = 0.0149). Moreover, the protective effect of SIINFEKL-NP treatment was associated with significantly reduced liver-infiltration of pathogenic OT-I T cells (42.2% vs. 77.3% of CD8; P = 0.0159). Furthermore, upon SIINFEKL-NP treatment, OT-I cells retrieved from the livers of K14-OVAp mice manifested a tolerized phenotype with significant up-regulation of PD-1 (MFI 8708 vs. 5600, P = 0.0079) and down-regulation of IFN gamma (76.8% vs. 49.8%, P = 0.0012) and granzyme B (71.9% vs. 28.4%, P = 0.0079).

Conclusions Our study provides proof-of-concept that LSEC-targeting NPs could function as antigen-specific therapeutic platform providing treatment for CD8 T cell-driven autoimmune responses, in addition to CD4 T cell-driven autoimmune diseases, as previously shown.

5.2 NK cells regulate LSEC to promote the HBV-specific T cells response

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Introduction It is unclear whether NK cells could regulate the function of liver sinusoidal endothelial cells (LSECs). Our previous study have indicated that activation of NOD1 signaling in the liver could enhance T cell response in a LSEC dependent manner and promote the recruitment and activation of NK cells in liver. Therefore, we hypothesized that the maturation of LSEC was dependent on NK cells.

Objectives Exploring whether intrahepatic NK cells could regulate the maturation of LSECs, and its effects and mechanism on HBV-specific T cells response.

Materials & methods The chronic HBV replication model was established by hydrodynamic injection (HI) of pAAV/HBV1.2 plasmid to male C57B6/L, CXCR3-/- or Rag1-/- mice. NOD1 ligand (diaminopimelic acid, DAP) was injected to mice by the same way. NK cells depletion was achieved by administration of anti- asialo-GM1. Purified intrahepatic NK cells and LSECs were cocultured at a 1:5 ratio with or without DAP presence. The phenotype of LSECs and HBV specific T cell response were detected by FACS.

Results:

1. NK cells depletion significantly downregulated the costimulatory molecules and adhesion molecules on LSEC while upregulated the inhibitory molecule; thus impaired the maturation of LSEC function and the anti-HBV effect of DAP.
2. Transfer CD8+T cells into NK cells depleted Rag-/- mice can"t restore the anti-HBV effects.
3. cNK cells were recruited into liver and secreted more IFN-γ comparing the NS control after the treatment of DAP.
4. NK cells was recruited into liver through CXCR3 axis and the anti-HBV effects of DAP also depended on this axis.
5. Intrahepatic NK cells purified from naïve mice promoted the maturation of LSEC function, while NK cells from HBV mice impaired its effect in vitro, indicating that HBV impaired NK cells function.
6. NK cells promote LSEC maturation through both soluble cytokines and direct contact.

Conclusion We demonstrated a crucial role of NK cells for LSEC maturation and activation as well as HBV-specific T cells response during DAP treatment in CHB mice.

5.3 TOX expression on HBV specific CD8+ T cells is linked to clinical stage of chronic HBV infection

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DOI 10.1055/s-0039-3402203

T-cell exhaustion or dysfunction is a common feature of chronic viral infections such as a chronic HBV infection. The mechanisms controlling the differentiation of exhausted T cells are poorly understood. Recently, the transcription factor and epigenetic modifier TOX was identified as a master regulator of the T-cell exhaustion program in mice. However, the role of TOX in T-cell responses to HBV and different human viral antigens remains unclear.

To address this important question, we analyzed the phenotype and function of HLA-A*02:01 restricted virus-specific CD8+ T cells from 23 HBeAg negative and 7 HBeAg positive chronically HBV-infected patients, as well as patients chronically infected with HCV, HIV, and healthy controls using flow cytometry and high-parametric mass cytometry.

We found that TOX expression on virus-specific CD8+ T cells was strongly increased in persistent viral infections. Interestingly, TOX was significantly higher expressed on HBV-specific T cells facing high levels of antigen isolated from HBeAg positive patients compared to patients with HBeAg negative infection (inactive carrier) indicating that TOX is linked to the different clinical stages of HBV infection and to high antigen load. In agreement with this assumption, we found that virus-specific CD8+ T cells obtained from chronically HCV- and HIV-infected patients with high viral loads also expressed high levels of TOX. Functional analysis linked TOX to a diminished polyfunctionality of exhausted HBV-specific CD8+ T cells suggesting a TOX-dependent programming of CD8+ T-cell dysfunction. Despite these clear links of TOX to severe T-cell exhaustion, we also observed a high TOX expression on memory CD8+ T cells, in particular senescent cells with irreversible arrest of cell proliferation and cell function in patients with chronic viral infection but also healthy individuals. Indeed, differential patterns of Eomes, T-bet and TCF1 co-expression with TOX were transcriptionally linked to exhaustion vs. senescence programs.

In sum, these results reveal a context-dependent role for TOX tied into differential antigen-dependent exhaustion and senescence programs in humans. The links between TOX and differential exhaustion of HBV-specific T cells during different clinical stages of HBV infection has implications for immunotherapeutic approaches in chronic HBV infection.

Poster Visit Session IV Tumors
Saturday, February 15, 2020, 8:30 am – 09:15 am, Lecture Hall P1

4.4 Hepatic activation of FOXO3 regulates mTORC2-Akt and enhances oxidative damage-associated hepatocellular carcinogenesis

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Background Hepatocellular carcinoma (HCC) is the most prevalent primary liver cancer, accounting for 80 – 90 % of cases. Mutations are commonly found in the signaling regulating the PI3K/Akt pathway, leading to oncogenic cell proliferation and survival. Key transcription factors that are negatively regulated downstream of PI3K/Akt are members of the forkhead box O family (FOXO). FOXOs were initially considered as tumor suppressors by inducing cell cycle arrest and apoptosis. However, there is increasing evidence showing that FOXOs, especially FOXO3, can support tumorigenesis.

Methods To understand the roles of FOXO3 in liver tumorigenesis and hepatocarcinogenesis, we analyzed HCC patient specimens and also established a doxycycline-regulated transgenic mouse model with hepatocyte-specific FOXO3 expression in a constitutively active form.

Results We found that FOXO3 protein is significantly overexpressed and activated in livers of HCC patients. Hepatic activation of FOXO3 induced extensive hepatic damage and elevated gene expression of several HCC-associated factors. Furthermore, FOXO3 expression enhanced hepatotoxicin-induced tumorigenesis. Mechanistically, FOXO3 activation caused oxidative stress and DNA damage and triggered positive feedback-loop for Akt activation as well as mTORC2 activation. Interestingly, FOXO3 activated not only reactive oxygen species (ROS)-promoting pathways, but also ROS-eliminating systems, which can be associated with the activation of the pentose phosphate pathway.

Conclusions FOXO3 is a master regulator of ROS. On one side, FOXO3 supports in protecting from ROS and may avoid cellular crisis but FOXO3 can also promote ROS signaling on the other side and support hepatocellular carcinogenesis.

4.5 Expression and Function of Fibroblast Growth Factor 9 in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is closely linked to hepatic fibrosis. The activation of hepatic stellate cells (HSC) is the key event of liver fibrosis and activated HSC are major players in hepatocarcinogenesis. Following activation, HSC produce fibroblast growth factors (FGFs) to promote liver regeneration. FGF signaling plays a major role in development, differentiation and wound healing. The FGF family comprises 22 proteins that can be classified into paracrine, intracrine and endocrine factors. FGFs signal through transmembrane tyrosine kinase FGF receptors (FGFRs). Overexpression of FGFRs contributes to HCC development and progression. However, the expression and tumorigenic effects of FGFR-ligands in HCC are largely unknown.

The aim of this study was to elucidate the role of paracrine FGFs in the HSC-HCC crosstalk with focus on FGF9.

Methods and Results Expression analysis of the fifteen paracrine FGFs revealed that FGF9 was expressed by activated human HSC while no FGF9 expression was detectable in human HCC cell lines (Hep3B, HepG2, Huh7, PLC). Immunofluorescence stainings of human HCC tissues showed co-localization of FGF9 and alpha-smooth muscle actin (alpha-sma), a characteristic marker of activated HSC. Fitting to this, FGF9 expression significantly correlated with alpha-sma expression in human HCC tissues indicating activated HSC as cellular FGF9 sources. Importantly, high expression levels of FGF9 significantly correlated with poor patient survival. Stimulation with recombinant FGF9 (rFGF9) induced ERK- and JNK-phosphorylation in HCC cells. In functional *in vitro* analysis, rFGF9 significantly increased proliferation, colony formation and migration of HCC cells. Furthermore, stimulation with rFGF9 significantly reduced the efficacy of sorafenib to inhibit proliferation and to induce apoptosis in HCC cells. Protumorigenic effects of FGF9 on HCC cells were almost completely blunted by the FGFR1/2/3 inhibitor BGJ398, while the selective FGFR4 inhibitor BLU9931 had no significant effect.

Summary and conclusion Stroma-derived FGF9 enhances the tumorigenicity and sorafenib resistance of HCC cells and FGF9 overexpression correlates with poor prognosis in HCC patients. Herewith, FGF9 appears as potential prognostic marker and novel therapeutic target in HCC.

4.6 Dysregulation of Hippo/Yap signaling induces chromosome instability in intrahepatic cholangiocarcinoma

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Background Cholangiocarcinoma (CC) is a highly aggressive tumor of the biliary tract and the second most frequent primary liver cancer. Intrahepatic cholangiocarcinoma (iCC) is the least common subtype, representing 10 – 25 % of all cases. The significance of the Hippo pathway effectors Yap (yes-associated protein) and TAZ (WWTR1; WW domain containing transcription regulator 1) was already demonstrated in hepatocellular carcinoma; however, their relevance in iCC is not well understood.

Methods Transcriptome data derived from 16 tumor samples (iCC) and seven corresponding nontumorous tissues were analysed for the expression levels of YAP and TAZ as well as markers of chromosomal instability (CIN). iCC tissue-microarrays (TMA) containing non-tumorous liver tissues (n = 11), non-tumorous gall bladder tissues (n = 5) and iCC tissues (n = 310) were stained immunohistochemically against YAP, MCM2 (minichromosome maintenance complex component 2), p53, pH2AX1 (phospho-histone H2AX) and the proliferation marker Ki-67. CIN signature genes (n = 16) and cell viability were analysed in iCC cell lines (HUCCT-1 and Huh-28) after siRNA-mediated silencing of YAP/TAZ.

Results Gene expression profiling showed that both YAP and TAZ gene expression was significantly increased in human iCC tissues in comparison to normal liver tissue. Moreover 22 out of 25 of CIN25 signature genes were increased significantly in tumor tissues. At the protein level, 43 % of all iCCs showed a moderate to strong nuclear expression of YAP, while no positivity was detected in nontumorous liver tissue. Equally, MCM2 (69 %) p53 (81 %), and the CIN marker pH2AX1 (26 %) were clearly expressed in iCC tissue. Importantly, a significant correlation between the nuclear enrichment of YAP and MCM2 ($rS = 0{,}436$; $p < 0{,}001$), p53 ($rS = 0{,}326$; $p < 0{,}001$) and pH2AX1 ($rS = 0.341$; $p < 0.001$) was detectable. In vitro, YAP/TAZ inhibition diminished

cell viability over time. Of note, a significant reduction of known YAP target genes and CIN signature genes was detectable.

Conclusion Dysregulation of the Hippo/YAP/TAZ signaling axis is frequently observed in iCC cells. In this tumor entity, YAP and TAZ may contribute to cancer initiation through the induction of chromosomal instability.

4.7 Quantitative LC-MS-based shot gun proteomics identifies deregulated proteins in gallbladder carcinoma

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DOI 10.1055/s-0039-3402207

Gallbladder carcinoma (GBC) is a rare and understudied cancer entity. Radical surgery is the only potentially curative treatment option but due to late diagnosis few patients are eligible and 2-year survival rates of unresectable GBC is less than 10%. Therefore, the development of new treatment options, including targeted therapy for GBC is required to improve patient outcome.

We performed quantitative LC-MS-based proteomics of a total of 18 FFPE tumors and 5 healthy gallbladder specimens from a German GBC cohort. Quantitative proteomic analysis was performed and revealed differentially expressed proteins, which were further subjected to pathway analysis. Candidate genes representing potential tumor suppressors were selected from the significantly altered pathways. The role of these candidate genes in proliferation, migration and clonogenicity was investigated in GBC cell lines to evaluate their specific function and their potential as future treatment targets.

In the investigated human samples, we detected 611 proteins with a significant difference (adj. p-value < 0.05) between long and short surviving patients. Among the enriched pathways in patients with worse survival are the cellular defense response and regulated exocytosis and downregulated pathways are the extracellular matrix organization and cell/biological adhesion. We also found 1766 proteins to be differentially expressed between healthy gallbladder and GBC (adjusted p-value < 0.05). Interestingly, multiple pathways for RNA processing and inflammatory response were upregulated, whereas cytoskeleton organization and biological/cell adhesion were down regulated.

In order to identify potential tumor suppressor candidates, we selected genes of the significantly affected downregulated pathways with at least 4-fold decrease in tumors. This led to the identification of FHL1 which is strongly downregulated in GBC tumor samples, compared to healthy gallbladder (fold difference: 8.6, FDR = 0.008). Cell lines were infected with an inducible lentivirus to study the biological functions of FHL1. Expression of FHL1 in NOZ and G-415 cells significantly reduced cell viability supporting a tumor suppressive role of FHL1 in GBC.

Thus, proteomic profiling of a clinicopathologically well-characterized German GBC cohort identified multiple differentially expressed proteins and tumor-relevant pathways. Functional validation confirmed the tumor suppressive function of FHL1.

4.8 Influence on hepatocellular carcinoma energy metabolism by pharmacological inhibition of the epigenetic modifier LSD1

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Question Epigenetic alterations play an important role in carcinogenesis. The lysine demethylase 1 (LSD1), regulating transcription by demethylation of histone 3 at lysine 4 and 9, is an epigenetic modifier, which is overexpressed in many cancer types including hepatocellular carcinoma (HCC). Our recent studies have demonstrated that LSD1 inhibition impairs cell growth and invasion. Hereby, we now addressed the question which mechanistic links are affected by LSD1 inhibition using the novel LSD1 inhibitor HCI-2509.

Methods Ultra-deep RNA-sequencing of LSD1 inhibited and non-inhibited HCC cells (Huh7, HepG2, Hep3B) was followed by comprehensive pathway analysis (IPA). Divergent expression in response to LSD1 inhibition was then verified by quantitative PCR. Changes of the mitochondrial membrane potential were investigated by confocal microscopy and flow cytometry. Metabolic and respiratory changes were further determined by the Seahorse platform. Besides, TCGA data (*National Cancer Institute*) from HCC patients were evaluated with respect to differential expression of LSD1 targeted metabolic genes in malignant versus healthy liver samples.

Results Gene expression profiling followed by pathway analysis of hepatoma cell types revealed a prominent dysregulation of genes involved in the cell cycle control and mitochondrial function after pharmacological LSD1 inhibition using HCI-2509. Especially, genes encoding subunits of the mitochondrial respiratory complex were repressed. In agreement, the mitochondrial membrane potential was decreased after LSD1 inhibition and metabolic analysis revealed reduced ATP production and a low respiratory capacity. Loss of mitochondrial function is accompanied by reduced expression of enzymes involved in mitochondrial metabolism, but with a pronounced increase of mitophagy sensors' expression. Furthermore, TCGA data from HCC patients pointed to a distinct difference of the expression of LSD1 targeted metabolic genes in tumor samples compared to non-transformed tissues.

Conclusions Our findings show that LSD1 inhibition leads to an impaired cellular respiration caused by a strong repression of the electron transport chain complex I and the overexpression of mitophagy inducers. Hence, our data emphasizes the value of pharmaceutical LSD1 inhibition as an antineoplastic therapeutic option, affecting HCC cell proliferation not only by cell cycle interruption, but also by mitochondrial dysfunction and energetic restriction.

4.9 KRAS-mutated intrahepatic cholangiocarcinoma shows preferential sensitivity towards PARP-1-based interventions

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Intrahepatic cholangiocarcinoma (iCCA) is a highly aggressive malignancy of the biliary tract and characterized by its profound genetic heterogeneity. Activating KRAS mutations rank among the most abundant genetic alterations and the presence of this genetic alteration is associated with early recurrence, poor therapeutic response and reduced overall survival in iCCA. In preliminary experiments we found that inhibition of DNA damage response protein Poly (ADP-ribose) polymerase 1 (PARP-1) reduces survival of KRAS-mutated iCCA cell lines; though the exact mechanisms of PARP-1 in cholangiocarcinogenesis are still unknown.

CRISPR/Cas9-mediated knockout and treatment with PARP-1-inhibitor AZD2281 were conducted in several KRAS-mutated and non-mutated iCCA cell lines as well as in patient-derived primary cells. Assessment of PARP-1 knockout and inhibition on tumorigenic potential was analyzed by colony

and sphere formation. RNA Sequencing was employed to further analyze underlying molecular pathways.

A significant upregulation of PARP-1, as well as enrichment of a gene set related to PARP-1 activation, was observed in iCCA tissue and RNASeq data compared to control, indicating a potential role of a PARP-1-signature in cholangiocarcinogenesis. Interestingly, a higher PARP-1 expression was also demonstrated in KRAS-mutated compared to non-mutated cell lines. Consistently, knockout of PARP-1 showed a preferential effect in KRAS-mutated cell lines and led to a 40 – 45% reduction in colony and sphere formation and sensitized KRAS-mutated cells towards DNA double-strand break-inducing agents. In addition, KRAS-mutated cell lines showed a significantly higher sensitivity to treatment with the PARP-1-inhibitor. RNA Sequencing analysis revealed differential expression of DNA damage response pathways as well as other cellular pathways known to be affected by PARP-1, e. g. apoptosis.

These investigations establish a therapeutic role for PARP-1 inhibition in iCCA. The preferential sensitivity of KRAS-mutated cell lines for PARP-1-based interventions suggests a potential interaction and might be clinically relevant.

4.10 The TAZ target gene ITGAV regulates invasion and positively feedbacks on YAP and TAZ in liver cancer cells

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The Hippo signaling pathway is an important regulator of cell proliferation and organ growth. Its effector proteins yes-associated protein (YAP) and WW Domain Containing Transcription Regulator 1 (WWTR1; TAZ) have been described as potent oncogenes in various cancer entities, promoting tumor initiation and progression. In hepatocellular carcinoma (HCC), YAP activation is linked to tumor onset and poor patient prognosis. However, if and how YAP and TAZ contribute to liver tumorigenesis *via* common and exclusive molecular mechanisms is poorly understood.

Using RNA*interference*, we illustrated that YAP and TAZ individually support HCC cell viability and migration, indicating similar molecular functions of YAP and TAZ. However, additive effects of YAP and TAZ on cell invasiveness were observed, suggesting that both factors may facilitate effects via partially independent mechanisms. To characterize responsible downstream target genes, we performed comprehensive expression profiling, which revealed partly overlapping YAP/TAZ target genes as well as exclusively regulated genes. Focusing on genes important for cell mobility, we identified Integrin-αV (ITGAV) as a novel TAZ-specific target gene. Using transcriptome data from HCC patients we showed that ITGAV is overexpressed in HCC tissues and linked with poor clinical outcome. Indeed, ITGAV expression correlated with TAZ expression and YAP/TAZ target genes in human HCC tissues. ITGAV contributed to tumor cell migration and invasion possibly by altering actin stress fiber assembly. As YAP and TAZ are described mechano-transducers, we showed that perturbation of ITGAV by using either a genetic approach or the specific ITGAV inhibitor cilengitide not only led to a reduction of actin stress fiber formation but also to diminished YAP/TAZ protein levels in the nucleus.

These data describe a novel downstream mechanism of the Hippo pathway in HCC cells, which is regulated by TAZ and ITGAV and that feedbacks on YAP/TAZ activity via dynamic cytoskeletal processes. This mechanism may represent a therapeutic target structure since it contributes to signal amplification of oncogenic YAP/TAZ signaling in hepatocarcinogenesis.

4.11 The desmosomal cadherin desmoglein-2 regulates the Hippo pathway effector yes-associated protein (YAP) in liver cancer

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Parenchymal hepatocytes are highly organized in a 3-dimensional network while loss of cell-cell contacts and cellular polarity are key feature in chronic liver damage and hepatocarcinogenesis. In this context, cadherins are key factors in the formation of junctional structures and maintain cell polarity in hepatocytes. Previous data illustrate that the Hippo pathway is a regulator of cell autonomous organ growth due to its ability to sense extracellular information, thereby negatively regulating the oncogene yes-associated protein (YAP). However, if and to which extent reorganization of junctional structures impair hepatocellular functionality in a Hippo pathway-dependent manner is not well understood. In this project we aim to understand how junctional proteins may affect liver cancer development via the Hippo/YAP axis.

To identify junctional factors with potential impact on the Hippo/YAP pathway, expression data from primary human hepatocellular cancer (HCC) tissues and respective surrounding liver tissues were analyzed ($n = 247$; Roessler et al., 2010). Several junctional proteins were significantly dysregulated in HCC tissues (e. g. Cadherin-2, Desmoglein-3). Similarly, the desmosomal cadherin desmoglein-2 (DSG2) was overexpressed in HCCs ($p < 0.001$) and its expression correlated with shorter patient survival ($p = 0.066$). Importantly, DSG2 expression significantly associated with the expression of a YAP-dependent gene signature ($r = 0.448$, $p < 0.001$). Indeed, Western blotting and immunofluorescence analyses revealed that siRNA-mediated DSG2 reduction led to a nuclear export of YAP in HCC cell lines. In addition, the concentration of typical YAP target genes was diminished at the transcript and protein levels (e. g. cysteine rich angiogenic inducer 61, CYR61). Functionally, DSG2 inhibition phenocopies reduced HCC cell viability.

These data suggest that the reorganisation of desmosomal structures affects YAP activity and may represent a novel upstream regulator of the Hippo pathway in liver tumorigenesis.

4.12 Strategies to identify oncogene-dependent long non-coding RNAs (lncRNAs) in liver cancer

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The Hippo signaling pathway plays a crucial role in human hepatocarcinogenesis. Its key signaling protein, *yes-associated protein* (YAP) and its paralog *WW domain containing transcription regulator 1* (WWTR1; synonym: TAZ) are highly expressed in *hepatocellular carcinoma* (HCC) and display high therapeutic potential. Therefore, noninvasive strategies to identify patients suitable for Hippo pathway-directed therapy are needed. Liquid biopsy testing has shown great promise for personalized medicine. Especially, the detection of long non-coding RNAs (lncRNAs) has proven itself to be a valuable method to diagnose and classify cancer. In this project, we aim to identify YAP/TAZ-regulated lncRNA networks, which could serve as biomarkers for YAP/TAZ activation in HCCs.

In order to identify YAP/TAZ-regulated lncRNAs, two complementary strategies were applied: a sequencing-based approach of genetically manipulated HCC cells (HLF cells) and a bioinformatic-based approach using ChIP-seq data. While the former aimed to identify significantly downregulated lncRNA candidates upon YAP/TAZ silencing, the latter was applied to detect lncRNAs harboring transcription factor binding sites of the TEA domain family members (TEAD), the main interaction partners of YAP and TAZ. Total RNA-sequencing after siRNA-mediated YAP/TAZ inhibition led to a significant reduction of 2708

mRNAs including known YAP/TAZ target genes such as CTGF and 119 lncRNAs (p-value < 0.05). For the bioinformatic approach, we utilized ChIP-Seq data of TEAD 1, 3 and 4 from the ENCODE database, extracted the peaks, annotated them to the nearest gene and filtered for lncRNAs (n1 = 949, n3 = 1992 and n4 = 324, respectively). Comparing the NGS data with the results from the bioinformatic approach revealed 49 significantly downregulated lncRNAs (fold change < 0.75), of which 19 had predicted binding sites of at least two TEAD family members. Selected candidate lncRNAs such as *shieldin complex subunit 2 pseudogene 2* (SHLD2P3) were confirmed in independent experiments.

In summary, we developed a novel and stringent strategy combining RNA-sequencing and bioinformatics to identify YAP/TAZ regulated mRNAs and lncRNAs in liver cancer. This approach might be a valuable tool to detect biomarkers that characterize HCC patients with aberrant YAP/TAZ activity.

4.13 Histone deacetylases (HDAC) expressions in HCC and functional effects of HDAC inhibitors in liver cancer cells

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Histone deacetylases (HDACs) comprise in humans currently 18 members divided in 4 classes. Histone deacetylase (HDAC) inhibition (HDACi) is emerging as a promising therapeutic strategy. However, most pharmacological HDACi unselectively block different HDAC classes and their molecular mechanisms of action are only incompletely understood.

The aim of this study was to systematically analyse the expressions of different HDAC classes in HCC cells and tissues and to functionally analyze the effect of the HDACi suberanilohydroxamic acid (SAHA) and trichostatin A (TSA) on tumorigenicity of HCC cells.

Methods and Results Gene expression of all HDAC classes was significantly increased in human HCC cell lines (Hep3B, HepG2, PLC, HuH7) compared to primary human hepatocytes (PHH). Analysis of HCC patient data showed increased expression of several HDACs in HCC tissues compared to non-tumorous liver. However, there was no unified picture of regulation in three different HCC patient data sets and we observed a strong variation in gene expression of different HDACs in tumorous as well as non-tumorous liver. Still, there was a strong correlation in the expression of HDAC class IIa (HDAC4, 5, 7, 9) as well as HDAC2 and 8 (class I) and HDAC10 (class IIb) and HDAC11 (class IV) in HCC tissues of individual patients. This might indicate a common mechanism of the regulation of these HDACs in HCC. TCGA (The Cancer Genome Atlas) data set analysis revealed that HDAC4, HDAC7 and HDAC9 as well as HDAC class I members HDAC1 and HDAC2 significantly correlated with patients' survival. Furthermore, we observed that SAHA and TSA reduced proliferation, clonogenicity and migratory potential of HCC cells. SAHA but not TSA induced features of senescence in HCC cells. Additionally, HDACi enhanced the efficacy of sorafenib in killing sorafenib susceptible cells. Moreover, HDACi reestablished sorafenib sensitivity in resistant HCC cells. As potential underlying mechanisms of the combined HDACi and sorafenib effects we identified enhanced expression of the cytochrome CYP2E1.

Summary and conclusion HDACs are significantly but differently increased in HCC, which may be exploited to develop more targeted therapeutic approaches. HDACi affect different facets of tumorigenicity of HCC cells and appears to be a promising therapeutic approach alone or in combination with sorafenib.

4.14 Cancer stem cells as significant drivers of sorafenib resistance in hepatocellular carcinoma

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Development of chemoresistance is frequently observed in the majority of HCC patients. Evidence suggests that cancer stem cells (CSCs) may contribute to the acquisition of resistance in many solid tumors, but their exact role in this process for HCC remains to be defined.

Here, we evaluate the importance of TICs in the development of resistance and relapse formation after exposure to sorafenib in HCC and define concomitant adaptive molecular changes.

Four HCC cell lines and two primary HCC isolates were exposed to sorafenib for a total of 14 days. The treatment effects on CSCs were estimated by sphere forming capacity *in vitro* and tumor-initiating potential *in vivo*, as well as the side-population (SP) approach. Expression of key oncogenic and CSC markers, such as EpCAM, CD133 and ABCG2 transporter, were assessed by qRT-PCR and flow cytometry. Whole transcriptome analyses identified potential targets which were validated by western blot and administration of specific inhibitors.

Treatment effectively reduced oncogenic properties in all investigated HCC cells. However, sustained anti-proliferative effect after treatment was observed in three cell lines, while initial treatment effect in other lines was followed by rapid re-growth thereby mimicking responses observed in patients. While anti-oncogenic effects in sensitive cell lines were associated with significant reduction in sphere forming capacity, CSC marker EpCAM as well as SP cells, resistant cells showed increased CSC properties. Acquired resistance to the drug uniformly developed in cell lines suggesting that common molecular mechanisms might be operative. Adaptive molecular changes involved signaling pathways associated with cell survival, proliferation and cell cycle regulation (RAS, AKT, MYC, P53). Furthermore, the resistant cell lines showed compensatory upregulation of key oncogenic molecules such as EGFR, multidrug resistance ABC transporters as well as YAP. Conclusively, combined treatment including sorafenib and specific YAP inhibitor showed beneficial effects in resistant cell lines which resulted in complete response to the therapy.

Our model recapitulates features of drug resistance observed in human HCC patients. Resistance to sorafenib therapy might be fueled by transient expansion of CSCs. Therefore, specific targeting of CSCs as well as pro-oncogenic compensatory signaling pathways might be an effective therapeutic strategy to overcome resistance in HCC.

4.15 Improvement in median OS in patients with advanced HCC and strict eligibility criteria receiving Sorafenib

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Introduction *Llovet et al.* demonstrated within the SHARP-trial that the median overall survival was nearly 3 months prolonged for patients with advanced hepatocellular carcinoma (HCC) and treatment with sorafenib compared to placebo. It is unclear whether patients with modified eligibility criteria (MEC)

benefit as well as patients with strict eligibility criteria (SEC) according to the SHARP-criteria.

Methods and Patients Within this retrospective single center analysis we initially reviewed electronic records of 987 patient diagnosed with HCC, who were treated between January 2008 and October 2017. Finally, 293 patients received sorafenib and were eligible for final analysis. Data of risk-factors, liver function, BCLC stage, ECOG, laboratory values, radiological findings, treatment modalities, and follow-up were recorded until 31.12.2017. Primary outcomes were median overall survival (OS) of patients with MEC and SEC according to the SHARP-criteria, as well as the safety-profile during treatment. Furthermore, a secondary endpoint was median OS depending on risk-factors.

Results The median OS was prolonged with 12.0 months in patients with SEC compared to 10.7 months in the SHARP-trial itself. Even patients with MEC, representing real-life practice, reached a median OS of 10.0 months without disadvantages concerning the safety of the drug. The incidence of drug-related adverse events (mainly grade 1 or 2) was 80.2% in patients with MEC and only 70.0% in patients with SEC compared to 80.0% in the SHARP-trial. Major adverse events included fatigue (36%), diarrhea (35%), and hand-foot skin syndrome (31%). A trend to prolonged median OS was demonstrated in patients with chronic hepatitis C (15.0 months in the MEC-cohort and 25.0 months in the SEC-cohort) compared to alcohol- or hepatitis b (9.0 months and 10.0 months)-related underlying liver disease ($p = n.s.$).

Conclusion Patients with advanced HCC do benefit from systemic treatment with sorafenib under real life conditions reaching a similar outcome. Patients with SEC, according to the SHARP-criteria, displayed an even prolonged median OS, which goes along with previous data from several clinical phase III trials, in which patients receiving sorafenib in a control arm, also demonstrated prolonged OS compared to the SHARP-trial.

4.16 HILPDA is upregulated, predicts prognosis and promotes cancer progression in hepatocellular carcinoma

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Aim Liver cancer is the second most common cause of cancer-related death worldwide, with hepatocellular carcinoma (HCC) being its predominant form. There is an urgent need to identify new prognostic markers to determine prognosis and select specific therapies. Hypoxia-inducible lipid droplet-associated protein (HILPDA) is induced in different cancer types, predicts worse prognosis and inhibits fatty acid oxidation, but its role in HCC, a tumor that frequently shows increased amounts of lipid droplets, has not been investigated so far.

Methods In order to investigate HILPDA-expression in HCCs in a large cohort of patients by immunohistochemistry, we established an HCC tissue microarray of 574 patients. Kaplan-Meier, Wilcoxon rank-sum test, log rank and univariate Cox's regression analysis and Spearman's rank correlation were used to determine associations between HILPDA-expression, patient survival, etiology of underlying liver disease and other clinical parameters. *In vitro*, proliferation rate and migratory activity were analyzed in Huh7 cells upon HILPDA overexpression.

Results We detected specific HILPDA immunoreactivity in over 95% of the HCCs, with strong HILPDA expression in about 5%, intermediate expression in about 25% and low expression in about 65% of primary HCCs. HILPDA was significantly induced in HCCs compared to surrounding non-neoplastic normal and cirrhotic liver tissue, and even further increased in lymph node metastases and recurrent HCCs. Increased HILPDA expression correlated with decreased survival (HR 1.31; 95% CI, 1.04 – 1.64, $p < 0.05$) and was associated with higher tumor grade, increased proliferation, and micro- and macrovascular invasion. Patients suffering from chronic hepatitis C showed increased HILPDA levels in HCC and non-neoplastic liver, whereas hemochromatosis and NASH were associated with lower HILPDA expression. We did not detect a significant association of HILPDA expression with other clinical parameters. *In vitro*, HILPDA overexpression resulted in an increased proliferation rate and increased tumor cell migration.

Conclusion HILPDA expression was induced in HCC and was further increased in relapses and lymph node metastases, as determined by immunohistochemistry. Higher HILPDA levels were associated with unfavorable prognosis, higher tumor grade and micro- and macrovascular invasion. Mechanistically, HILPDA overexpression led to increased tumor cell proliferation and increased migratory activity.

4.17 Expression and function of Epithelial Splicing Regulatory Protein 1 in hepatocellular carcinoma

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Epithelial-to-mesenchymal transition (EMT) plays a critical role in tumor progression and metastasis. The Epithelial Splicing Regulatory Protein 1 (ESRP1) belongs to the hnRNP family of RNA binding proteins and regulates alternative splicing of a number of genes associated with EMT. However, the expression and function of ESRP1 in hepatocellular carcinoma (HCC) is unknown.

The aim of this study was to analyze the expression, regulation and function of ESRP1 in HCC.

Methods and Results ESRP1 expression levels were analyzed in human HCC tissues and cell lines with RT-qPCR and Western blot analysis. ESRP1 expression varied significantly between different human HCC cell lines, with highest expression levels found in Hep3B compared with HepG2 and PLC cells. Fitting to this, comparison of ESRP1 expression levels in human HCC tissues revealed that there are "high" and "low" "ESRP1-expressors". Regarding the regulation of ESRP1 in HCC, we found that stimulation of HCC cells with recombinant TGFβ1, an EMT inductor, decreased ESRP1 expression. Conversely, siRNA-mediated knockdown of the EMT-inducer zink finger E-box binding homeobox 1 (ZEB1) significantly upregulated ESRP1 expression. Interestingly, siRNA-mediated ESRP1 knockdown decreased E-Cadherin expression and upregulated the expression of EMT markers N-Cadherin, Twist and ZEB1. Furthermore, ESRP1 knockdown caused a shift in the expression of splicing variants exon IIIb to exon IIIc of Fibroblast Growth Factor Receptors, which is known to be associated with a more aggressive phenotype of HCC. Moreover, ESRP1 knockdown significantly increased proliferation of Hep3B cells and induced ERK- and Akt-phosphorylation. Conversely, overexpression of ESRP1 in HepG2 and PLC cells decreased ERK- and Akt-activation.

Summary and Conclusion Decreased ESRP1 expression promotes tumorigenicity and EMT of HCC cells *in vitro*. Therefore, ESRP1 appears as potential prognostic parameter in HCC.

4.18 Expression and Function of Four-and-a-Half LIM-domain protein 2 (FHL2) in Hepatocellular Carcinoma

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The four-and-a-half LIM-domain protein 2 (FHL2) is an adaptor protein that can bind to different proteins, directing the functions of the protein complexes formed. FHL2 has been described to have multiple, often opposing functions in different cells and tissues. Also in different types of cancer, FHL2 has been associated with both pro- and anti-tumorigenic functions.

The aim of this study was to investigate the expression and role of FHL2 in hepatocellular carcinoma (HCC).

Methods and results FHL2 mRNA and protein expression were found to be significantly reduced in 4 human HCC cell lines compared to primary human hepatocytes. Also in human HCC tissues, FHL2 expression was significantly lower than in corresponding adjacent non-tumorous liver tissue. FHL2 expression was not increased in precancerous liver tissue of Mdr2 knockout mice, suggesting that the downregulation of FHL2 does not occur during HCC development but rather in advanced HCC. Analysis of HCC patient data showed that FHL2 expression was lower in high risk patient group (based on prognostic index) and correlated inversely with patients' survival. To get further insight into the role of FHL2 in HCC, we assessed HCC cells with siRNA-induced FHL2 suppression or adenoviral-mediated overexpression of FHL2 in functional in vitro assays. Neither knockdown nor overexpression of FHL2 had a significant effect on the proliferation of HCC cells. Furthermore, FHL2 suppression did not affect colony formation and colony growth in clonogenic assays. However, colony formation was significantly inhibited in FHL2 overexpressing cells. Furthermore, FHL2 suppression resulted in reduced expression of pro-inflammatory genes (IL-8 and ICAM-1) and an accumulation of p62 in HCC cells, indicative for impaired autophagy. Autophagy is known to affect the response to the multikinase inhibitor sorafenib, and interestingly, we observed that sorafenib treatment induced the expression of FHL2 in HCC cells. FHL2 depletion enhanced the sensitivity of HCC cells for sorafenib.

Summary and conclusion Expression analyses suggest an anti-tumorigenic role of FHL2 in HCC. However, functional analysis indicate both pro- and anti-tumorigenic effects of FHL2 in HCC cells and a complex role of FHL2 in combination with sorafenib treatment, which requires further investigation.

4.19 Macrophage Migration Inhibitory Factor promotes HCC progression *in vivo* via the receptor CD74

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Background and Aims Macrophage Migration Inhibitory Factor (MIF) is a proinflammatory cytokine with chemokine-like functions. Analysis of the GEPIA database revealed an increased expression of MIF in human hepatocellular (HCC) tumor tissue and high expression levels were associated with decreased survival in HCC patients. Referring to the MIF receptors, CD74 has been identified as an important target of MIF in malignant diseases. In this study, we analyze the functional role of MIF and its receptor CD74 in HCC progression *in vitro* and *in vivo* using an experimental murine model of HCC.

Methods HCC induction was induced in mice with a hepatocyte-specific *Mif* depletion (*AlfpCre*$^{+}$*Mif*$^{flox/flox}$ mice, *Mif*$^{\Delta hep}$), *CD74* deficient mice and respective control mice using the in DEN/CCl4 model. Tumor size and number were analysed as well as immune cell infiltration via FACS analysis. Next, qPCR of differentiation and inflammatory marker were performed from whole liver and tumor tissue. *In vitro*, we performed proliferation and apoptosis/cell death assays.

Results *Mif*$^{\Delta hep}$ mice showed a reduced tumor burden in the DEN/CCl4 model compared to their control littermates. Furthermore, tumors in *Mif*$^{\Delta hep}$ displayed a diminuished proliferation rate and higher grades of differentiation. The intrahepatic and intratumoral immune cell repertoire was unaltered between the genotypes. *In vitro*, MIF stimulates the proliferation of the HCC cell line Hepa1 – 6 in a dose-dependent manner and inhibits sorafenib-induced cell death as evidenced by decreased Annexin V- and TUNEL-staining. Both effects can be reversed using a CD74 neutralizing antibody. In line with these results, CD74 deficient mice are less susceptible to hepatic carcinogenesis developing fewer and well-differentiated tumors.

Conclusion In this study, we identified a pro-tumorigenic role of MIF in hepatic carcinogenesis. MIF directly promotes proliferation of HCC cells, is protective during therapy-induced apoptosis and impacts differentiation of carcinogen-induced tumors in mice. Furthermore, *in vitro and in vivo* studies implicate that these effects are mediated via the receptor CD74. In conclusion, the inhibition of the MIF/CD74 axis could present a promising target for the improvement of HCC-directed therapies.

4.20 Smad3 linker (S213 and S204) phosphorylation as indicators for the prediction of cholangiocarcinoma outcome

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Background TGF-β signal transduction initiates with receptor-mediated C-terminal phosphorylation of Smad2/3, subsequent complex formation with Smad4, and nuclear translocation for target gene regulation. Regulatory phosphorylation steps at serine/threonine residues in the Smad linker (L) domain create phosphorylated forms with distinct cellular functions, including transcriptional activity and proteasomal degradation. TGF-β signaling is impaired in several human tumors, however, the contribution of pSmad patterns to the development and progression of cancer is poorly understood. Here, we present evidence that Smad3 linker phosphorylation is a marker of cholangiocellular carcinoma (CCA).

Methods We examined healthy and tumorigenic liver tissues from CCA patients for the phosphorylation status of Smad3 at C-tail- and linker residues (S204, S208, and S213), using immunoblotting and immunohistochemistry with Smad phosphorylation site-specific antibodies. A kinase screen was performed in selected CCA cell lines using several inhibitors as well as a novel small molecule (designated as E738), targeting various kinases among others, GSK3 and MEK/ERK1/2. Readouts were Smad2/3 proteasomal degradation, Smad phosphorylation, cytotoxicity and apoptosis. We also included the kinase-inactive analogue of E738 (XC47) to further confirm the significance of kinase inhibition in the observed effects.

Results CCA patients and cell lines display variant Smad3 linker and C-terminal phosphorylation patterns. Tumorigenic areas from intrahepatic CCA patients present decreased pSmad3C and increased pSmad3L (S213 and 204) levels. In the CCSW1 cell line, we identified a counter-regulation of C-terminal versus linker phosphorylation, and GSK3, MEK/ERK1/2, and p38 MAPK as regulators of pSmad3L signalling. E738 was found to inhibit pSmad3L by Smad2/3 degradation, most likely through its kinase inhibitory function. Using a combination of inhibitors of the above kinases and E738, we observed synergistic effects on inhibition of pSmad3L protein levels, which coincided with reduced cellular viability and increased apoptotic cell death in CCA cell lines.

Conclusion We suggest that pSmad3L (S204) and pSmad3L (S213) are predominant in CCA and interfere with the cytostatic pSmad3C (S423/425)

signaling, inducing a malignant switch in TGF-β's responses. We show that this could be translated into a therapeutic strategy through the inhibition of kinases involved in Smad3 linker phosphorylation.

4.21 Mathematical modeling of the YAP/TAZ shuttling as a response to cell density, actin dynamics, and liver damaging drugs

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The Hippo pathway facilitates its biological function through the regulation of the transcriptional coactivators yes-associated protein (YAP) and WW domain containing transcription regulator 1 (WWTR1 or TAZ). However, both YAP and TAZ may respond differently to activating cues with respect to gene expression, their subcellular localization, and biological properties. To address the question, how YAP and TAZ deferentially respond to external stimuli, we utilized live-cell imaging, quantitative image analysis and mathematical modeling techniques.

Using lentiviruses, we stably transfected Hep3B cells with cerulean-tagged histon 2B (H2B), venus-tagged YAP and mCherry-tagged TAZ reporter constructs that allow to track nuclear-cytoplasmic shuttling of both factors via time-lapse microscopy. The time-resolved data was analyzed in a quantitative manner using Fiji. Image segmentation was performed with the Trainable Weka segmentation plugin, which performs object segmentation based on training of the machine learning algorithms. The output of the classification algorithm is probability maps that are thresholded, thus obtaining binary maps, which are used for the calculation of the nuclear to cytoplasmic ratio of YAP localisation. First, confirmatory experiments revealed that upon increasing cell density YAP clearly shifts from nucleus to cytoplasm. In addition, perturbation of the actin stress fibers by Latrunculin B (LatB) or Cytochalasin D (CytoD) under low cell density conditions led to the clear cytoplasmic enrichment in a dose-dependent manner. The liver-damaging drugs (acetaminophen) lead to slight nuclear enrichment of YAP. Using the measured data, we created an ordinary differential equation-based computational model, which describes Hippo pathway activation upon increasing cell density and perturbations of actin dynamics. In addition, we modeled the interactions between phosphorylated and unphosphorylated YAP and TAZ, actin, 14 – 3-3 and intracellular shuttling and degradation of YAP and TAZ.

In summary, we developed a tool that quantitatively describes the cellular YAP/TAZ response of hepatocellular cells on drugs and liver-damaging substances. Furthermore, this work for the first time shows, how the dynamic regulation of actin affects the subcellular localization of YAP and TAZ and thus activating Hippo pathway in a live-cell setting. In the future, we aim to investigate how Hippo pathway-directed cancer therapies predominantly affect YAP or TAZ.

4.22 Loss of Sirtuin6 induces expression of the cancer-related transmembrane protein TMEM45A in HepG2 cells

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Sirtuin 6 (SIRT6), a member of the sirtuin family of NAD+-dependent deacetylases is a key player in numerous cellular processes. The protein is crucial in aging-related pathways and cancer since it maintains genome stability and telomere integrity, promotes DNA repair and regulates metabolic homeostasis.

Loss or aberration of SIRT6 leads to epigenetic changes promoting a loosened chromatin state that ultimately results in major transcriptional deregulation. A cohort study with HCC patients confirmed the downregulation of SIRT6 in around 40 % of HCC tissues and gene expression related to SIRT6-deficiency was associated with a worse disease outcome.

In order to better understand the role of SIRT6 in HCC we generated SIRT6-deficient HepG2 hepatoblastoma cells by using the CRISPR/CAS gene editing system. Cell clones lacking SIRT6 showed increased acetylation levels of Histone 3 at Lysine 9, 18 and 27. Even though no difference in proliferation was observed, the knockout cells showed an increased colony formation capacity. By analysis of SIRT6-dependent gene expression signatures using RNA-Sequencing, we identified several cancer-related genes. Among them was the hypoxia-inducible transmembrane protein 45 A (TMEM45A) which is upregulated in different types of cancer. TMEM45A is mainly located in the transgolgi network and highly conserved in vertebrates. Although its biological function has not yet been elucidated, TMEM45A has been shown to be essential for chemoresistance of breast and liver cancer cells under hypoxic conditions.

We were able to confirm an elevated expression of TMEM45A in SIRT6-deficient HepG2 cells that was further increased under hypoxic conditions. Moreover we detected accumulation of acetylated HIF1α in SIRT6 knockout cells. Based on the direct interaction of SIRT6 and HIF1α we analyzed the molecular mechanism of TMEM45A expression and its functional consequences for liver cancer.

4.23 The role of ferroptosis in chronic liver disease

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Question The prevalence of non-alcoholic fatty liver disease, ranging from steatosis to non-alcoholic steatohepatitis (NASH), is increasing in developed countries. In some patients, progression towards cirrhosis and hepatocellular carcinoma (HCC) occurs. To present, the underlying mechanisms for disease progression remain incompletely understood. In NASH oxidative stress and lipid peroxidation constitute prominent features and may hence play a key role. Interestingly, accumulation of lipid peroxides can trigger ferroptosis, an iron-dependent mode of cell death. According to *in vitro* studies, acyl-CoA synthetase long-chain family member 4 (ACSL4) is an essential contributor to ferroptosis. In our study, we aimed to investigate the relevance of ferroptosis for disease progression using hepatocyte-specific ACSL4 inhibition in experimental models of chronic liver disease.

Methods: Primary hepatocytes from either wild-type (WT) mice or mice with hepatocyte-specific deletion of ACSL4 (ACSL4Δhepa) were treated with specific inducers (e. g., RSL3) and inhibitors (e. g., Liproxstatin-1) of ferroptosis. In addition, we compared disease development between WT and ACSL4Δhepa mice to investigate the role of ferroptosis. We used a high-fat diet (HFD) to trigger NASH and STZ (Streptozocin) with HFD as a NASH-HCC model.

Results Treatment of primary hepatocytes with RSL3 leads to increased cell death, which could be rescued by adding Liproxstatin-1 or by using ACSL4-deficient hepatocytes.

In our HFD-based NASH model, we did not find significant differences between WT and ACSL4Δhepa mice. Specifically, liver-to-body weight ratio, serum transaminase levels, and immune cell infiltration were not altered. In contrast, in our NASH-HCC model, inhibition of ferroptosis in hepatocytes augments chronic liver disease resulting in increased serum transaminase levels in ACSL4Δhepa mice. Importantly, while the overall tumor burden was not affected in ACSL4Δhepa mice, the number of smaller tumors was significantly increased.

Conclusion Our results demonstrate that primary mouse hepatocytes are susceptible to induction of ferroptosis and that this mode of cell death depends on functional ACSL4. Interestingly, ferroptosis seems to be a protective

mechanism during tumor initiation, by regulating lipid accumulation and cell death in hepatocytes. Therefore, activation of ferroptosis or inhibiting key molecules regulating cell death, can be a possible therapeutic treatment for human disease.

4.24 The NRF2/KEAP1 pathway in hepatocytes controls fibro- and carcinogenesis in chronic liver disease

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Question Oxidative stress has been considered as a conjoint pathological mechanism, and it contributes to initiation and progression of liver injury, fibrosis and carcinogenesis. The KEAP1 (Kelch-like ECH-associated protein-1)/NRF2 (erythroid 2-related factor 2) axis is a major regulator system of cellular redox balance. We investigated whether activation of the NRF2 pathway, due to KEAP1 deletion, affects the development of liver injury, fibrogenesis and HCC development in an inflammation driven murine HCC model.

Methods Hepatocyte specific NEMO ($NEMO^{\Delta hepa}$) knock-out mice were crossed with hepatocyte specific KEAP1 ($KEAP1^{\Delta hepa}$) knock-out mice to generate $NEMO^{\Delta hepa}/KEAP1^{\Delta hepa}$ mice. Primary hepatocytes as well as livers of all four genotypes were subjected to microarray analysis. Furthermore, liver injury, cell death, DNA damage, proliferation as well as liver fibrogenesis and HCC development were analyzed.

Results Microarray analysis of primary hepatocytes as well as livers revealed that hepatocyte specific KEAP1 deletion increased NRF2 target genes involved in glutathione metabolism and xenobiotic stress (e.g. HO-2, Nqo1). Furthermore the deficiency of one of the most important antioxidants, Glutathione (GSH) in $NEMO^{\Delta hepa}$ livers could be rescued by additionally deletion of KEAP1. As a consequence the activation of the NRF2 pathway resulted in reduced apoptosis in $NEMO^{\Delta hepa}/KEAP1^{\Delta hepa}$ livers compared to NEMOΔhepa livers. Microarray analysis of primary hepatocytes further revealed a dramatic downregulation of genes involved in cell cycle regulation and DNA replication in $NEMO^{\Delta hepa}/KEAP1^{\Delta hepa}$ compared to $NEMO^{\Delta hepa}$ primary hepatocytes. Of note, in livers of NEMOΔhepa/KEAP1Δhepa mice instead of hepatocytes, CK19+ cells are proliferating. Further validation in *in vitro* and *in vivo* experiments confirmed that the hepatocyte specific KEAP1 deletion protects from DNA damage. In aging mice, $NEMO^{\Delta hepa}/KEAP1^{\Delta hep}$a livers displayed decreased fibrogenesis, a lower tumor incidence, a reduced tumor number and decreased tumor size compared to $NEMO^{\Delta hepa}$ mice.

Conclusions Hepatocyte specific inactivation of KEAP1 in $NEMO^{\Delta hepa}$ livers attenuated apoptosis, DNA damage and hepatic fibrosis progression. Consequently, deletion of KEAP1 in $NEMO^{\Delta hepa}$ mice ameliorated HCC progression. Hence, KEAP1 is an attractive target to treat chronic liver disease.

4.25 Overexpression of tumor suppressor miR-198 in hepatoma cells leads to its spontaneous and active export via vesicles

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Background and Aim As a potent tumor suppressor, miR-198 expression is downregulated in various cancer types including liver cancer. Previous reports have shown that circulating miR-198 was detected in serum samples from patients with hepatocellular carcinoma. In the present study, we focused on molecular mechanisms of miR-198"s decrease in hepatoma cells.

Methods For conditional, doxycycline-induced miR-198 expression, the miR198 encoding sequence was cloned in a Tet-on vector system and stable, transgenic Huh7 and Hep3B derived hepatoma cell lines was generated. Vesicles were isolated from cell culture supernatants by ultracentrifugation. miR-198 of NA preparations, obtained from cells, supernatants, and from exosomal fractions, was quantified by qPCR. The miR-198 interacting proteome was analysed by pull down experiments followed by mass spectrometry and validated by immunoblotting. The impact of ubiquitination on the miR-198 vesicle export was studied functional transgenic mutants.

Results Under the Tet-on inducible system, after doxycycline treatment intracellular miR-198 was massively overexpressed within the first 8 hours. Interestingly, miR-198 overexpression was followed by a marked decrease in the next 36 hours. Meanwhile, in the supernatant there was a significant higher amount of extracellular miR-198. Isolation of exosomes from the supernatants demonstrated that extracellular miR-198 was enormously enriched in the exosomal fractions, characterized by the exosome markers, CD63 and HSP70. Both Ago2 and ubiquitin immunoprecipitation have shown that in HCC cells, miR-198 was interacting with Ago2 proteins and strongly associated with ubiquitin. Furthermore, treatment with various inhibitors proved vesicular release of miR-198 by ubiquitin-associated pathway.

Conclusion In hepatoma cells, intracellular expression levels of miR-198 were tightly modulated. The decrease of the tumor suppressor miR-198 during hepatocarcinogenesis is proposed to be due to, at least partly, vesicular release.

4.26 HELLS is an important p53 repression target in liver cancer

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The tumor suppressor protein p53 serves as an important barrier against the development and progression of liver cancer. Various p53 responses (e.g. cell cycle arrest, apoptosis, and senescence) are mediated by activation or repression of p53 target genes. To identify previously unrecognized p53 repression targets in liver cancer we used a large scale proteomics approach (LC/MS-MS, > 5000 quantified proteins) in HepG2 cells upon Nutlin-3a treatment (24 h). Among the most strikingly decreased proteins we found the chromatin remodeling enzyme "lymphoid specific" helicase HELLS (-2.64 log2 fold-change, q < 0.01). We could validate p53-mediated repression of HELLS by immunoblotting in HepG2 and Huh6 cells upon Nutlin-3a and Camptothecin (CPT) treatment, which was paralleled by a strong decrease in *HELLS* mRNA as measured by qRT-PCR. Confirming a p53-dependent regulation we could completely rescue decreased *HELLS* mRNA levels under Nutlin-3a treatment by siRNA mediated knockdown of *TP53*. Consistent with these findings we observed that HELLS is overexpressed in murine and human hepatocellular carcinoma (HCC) and that HELLS expression correlates with the p53 status.

Our data indicate that HELLS is an important transcriptional repression target of p53 and suggest that mutational or functional inactivation of p53 is an important event leading to HELLS overexpression in liver cancer. The underlying mechanism of p53-mediated repression of HELLS is currently being investigated.

4.27 Clinically applicable liver repopulation: the role of thymidine kinase and ganciclovir (GCV)

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Background and Aims Due to the shortage of transplantable donor livers, hepatocyte transplantation is an alternative therapy for inherited liver diseases patients. Nevertheless, the transplantation efficiency and long-term benefits of hepatocytes transplantation have to be improved. Thus, we applied thymidine kinase recombinant adeno-associated virus (AAV-TK) and ganciclovir (GCV) in order to improve the repopulation of host liver by transplanted hepatocytes.

Methods We evaluated the transfection efficiency of AAV-GFP and apoptosis induction of AAV-TK and GCV in Hepa 1 – 6 cells and freshly isolated primary mouse hepatocytes *in vitro*. We transplanted mouse primary hepatocytes after AAV-TK recombinant virus injection *in vivo,* which was followed by gradually increased dosages of GCV administration. The *in vivo* long-term transplantation efficiency and functional analyses were performed at the end of the study.

Results We found robust transfection efficiency and rapid apoptosis induced by AAV-TK and GCV in Hepa 1 – 6 cells and primary mouse hepatocytes *in vitro*. High transduction efficiency and gradually induced liver injury was observed *in vivo*. The extensive repopulated livers (> 40 %) revealed gradually recovered liver functions. Apoptosis of hepatocytes was restricted in recipient liver until month 6 after transplantation.

Conclusion Our findings reveal stable but non-fatal liver injury induced by AAV-TK and GCV system and increased repopulation of transplanted hepatocytes. Hence, AAV-TK/GCV may be suitable for increasing therapeutic efficiency of hepatocyte transplantation and may facilitate humanised mouse research.

4.28 TGFβ-activated kinase 1 (TAK1) is activated and predicts prognosis in hepatocellular carcinoma

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Aim Liver cancer is the second most common cause of cancer-related death worldwide, with hepatocellular carcinoma (HCC) being its predominant form. There is an urgent need to identify new prognostic markers to determine prognosis and select specific therapies. Tak1 is a master regulator of proinflammatory and prosurvival signaling pathways. Mice with a hepatocyte-specific deletion of Tak1 develop steatohepatitis, dysplastic nodules and HCCs, whereas on the other hand constitutive activation of Tak1 in mice also results in HCC development (Roh, J Gastroenterol. 2014, 49(2): 185 – 194). Despite detailed preclinical data, comprehensive studies on Tak1 expression in human HCC are lacking.

Methods In order to investigate Tak1 expression in HCCs in a large cohort of patients by immunohistochemistry, we established a tissue microarray (TMA) of HCCs of 574 patients who underwent tumor resection at the University Medical Center Mainz from 1998 to 2017. Kaplan-Meier, Wilcoxon rank-sum test, log rank and univariate Cox's regression analysis and Spearman's rank correlation were used to determine associations between Tak1 expression, patients' survival, etiology of underlying liver disease and other clinical parameters such as liver enzymes.

Results In the majority of HCCs, we detected Tak1 immunoreactivity predominantly in the nucleus. Tak1 was significantly induced in HCCs compared to surrounding non-neoplastic normal and cirrhotic liver tissue and was even further increased in distant metastases. Increased Tak1 expression in primary HCCs correlated with decreased survival (HR 1.27; 95 % CI, 1.01 – 1.59, $p < 0.05$) and was associated with macro- and microvascular invasion. HCCs and surrounding liver tissue from patients suffering from HBV- or HCV-infection, hemochromatosis, liver cirrhosis, alcoholic and non-alcoholic steatohepatitis showed significantly lower Tak1 levels compared to tissues from patients without a known underlying liver disease.

Conclusion Tak1 expression was induced in HCC and even further increased in distant metastases, as determined by immunohistochemistry, and higher Tak1 levels were associated with unfavorable prognosis and macro- and microvascular invasion. Interestingly, HCCs and surrounding liver tissue of patients without any known underlying liver disease showed increased Tak1 expression levels. Further *in vitro* and immunohistochemical studies are currently undertaken to elucidate the underlying mechanism.

4.29 Cis-E:N-Cadherin-Heterodimere als Charakteristikum von Tumoren des hepatopankreatobiliären Systems

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Einleitung Zell-Zell-Kontakte sind essentiell für Embryogenese und Organogenese multizellulärer Organismen, für Strukturerhalt und organspezifische Funktionen. In der Tumorigenese spielen sie eine große Rolle bei Invasion und Metastasierung, aber oft auch bei der Zelldifferenzierung. Es existieren zahlreiche Studien, in denen entsprechend einer epithelial-mesenchymalen Transition (EMT) eine Deregulation von E-Cadherin und Hochregulation von N-Cadherin in verschiedenen malignen Tumoren beschrieben und mit schlechter Prognose korreliert wurde. Wir konnten aber auch zeigen, dass N-Cadherin zusammen mit E-Cadherin ein Charakteristikum von z. B. Hepatozyten darstellt und diese in den hepatozytären "Adherens Junctions" in cis-E:N-Cadherin-Heterodimeren vorliegen.

Methodik E- und N-Cadherin wurden in Tumoren mit den entsprechenden Normalgeweben vergleichend proteinbiochemisch sowie in Tissue-Microarrays immunhistochemisch analysiert (hepatozelluläre Karzinome (HCC, 570), periphere und perihiläre intrahepatische Cholangiokarzinome (ICC, 250; PHCC 190), extrahepatische Gallengangskarzinome (ECC, 100), Adenokarzinome der Gallenblase (GBCA, 100), duktale Adenokarzinome des Pankreas (PDAC, 40), Colonkarzinome, primär und als Lebermetastasen (600), klarzellige Nierenzellkarzinome (ccRCC, 300) sowie Ovarialkarzinome (120) u. a.).

Ergebnisse In Normalgeweben kommt E-Cadherin in verschiedenen Spezies wie Mensch, Ratte, Maus, Rind und Schwein zusammen mit N-Cadherin in Hepatozyten und intrahepatischen Gallengängen vor, wohingegen in extrahepatischen Gallengängen, dem Gallenblasenepithel und den Ausführungsgängen des Pankreas immer weniger N-Cadherin synthetisiert wird, in Azinuszellen des Pankreas, in Magenfoveolen und Dünn- und Dickdarmepithel wurde kein N-Cadherin nachgewiesen. In abgeleiteten hepatopankreatobiliären Tumoren wie dem HCC und CC kommt E- und N-Cadherin jedoch weiterhin in cis-E:N-Cadherin-Heterodimeren vor, wobei auch immunfluoreszenzmikroskopisch in situ wie in entsprechenden Zellkulturen (z. B. HepG2, HuH7, PLC und Hep3B) an manchen Membranseiten auch nur E-Cadherin oder nur

N-Cadherin vorkommt. E- und N-Cadherin kommen auch in vielen PDAC vor, wohingegen in anderen Karzinomen E-Cadherin dominiert und nur geringe Mengen oder kein N-Cadherin nachweisbar ist.

Diskussion Cis-E:N-Cadherin-Heterodimere sind ein strukturelles Charakteristikum sowohl von normalen Zellen wie von Tumoren des hepatopankreatobiliären Systems und sind hier kein Zeichen einer EMT.

4.30 Distant metastases in patients with intrahepatic cholangiocarcinoma: Does location matter?

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Background Intrahepatic cholangiocarcinoma (ICC) is an aggressive tumor entity and distant metastases (DM) are common. However, studies investigating patterns and clinical relevance of DM are rare. Therefore, aim of this study was to analyze occurrence, location, and prognostic impact of distant metastases on median overall survival (mOS).

Methods and materials Between 1997 – 2018, 417 patients with histopathologically-confirmed ICC were referred to our center and identified out of a dedicated clinical database. Imaging studies, surgical and histopathological reports, and follow-up visits were retrospectively evaluated with respect to hepatic tumor burden and presence of DM.

Results Finally, 370 patients with histopathologically confirmed ICC could be included. Of these, 186 patients showed distant metastases, either initially (n = 59) or in the course of disease (n = 127). The most common metastatic sites were lung (n = 105), peritoneum (n = 81), and bone (n = 50). After detection of lung metastases, residual mOS was 5.3 months, after peritoneal metastases 4.5 months, and after bone metastases 4.4 months (p = 0.24). At the time of first metastatic occurrence, residual OS according to intrahepatic tumor burden of < 25 %, 25 – 50 %, and > 50 % was 6.5 months, 4.9 months, and 1.2 months respectively (p < 0.001). In multivariate hazard regression analysis, hepatic tumor burden, liver function, and subsequent treatment were significant predictors of survival.

Conclusion While distant metastases were associated with poor outcome, there was no significant difference between metastatic sites. However, outcome was heavily influenced by hepatic tumor burden. This supports efforts to try and achieve hepatic tumor control; besides chemotherapy interdisciplinary approaches including re-resection and intra-arterial therapy might be considerations on an individual basis.

4.31 Dual role of Transforming Growth Factor Beta1&2 during Tumor Promotion and Metastasis in Primary Liver Cancer

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Background Transforming Growth Factor Beta (TGF-β) belongs to a superfamily of cytokines that induces pleiotropic effects on different processes and cell types in the liver. While TGF-β signaling exerts tumor suppressive functions at pre-neoplastic and early tumor stages, cytostatic effects of TGF-β are often lost in progressed stages due to (epi-) genetic disruption of several members of the signaling pathway. Consequently, cancer cells display an epithelial-mesenchymal-transition (EMT) phenotype and acquire pro-metastatic properties.

Aims: To evaluate the effect of the TGF-β1 and TGF-β2 on (i) proliferative (ii) migratory and pro-metastatic properties of primary and established PLC cell lines.

Method Primary patient-derived (HCC & CCA) and established cell lines (PLC & HuCCT-1) were treated with TGF-β1 and TGF-β2 (1 ng/ml) for 72 hr. The effect of TGF-β1&2 on proliferation was determined by colony and sphere formation assays. Invasive and migratory properties were determined using the wound healing invasion assays. Next Generation Sequencing and RPPA (reverse phase protein array) was performed to explore differential transcriptomic and protein expression patterns across treatments.

Results Treatment with TGF-β1 and TGF-β2 led to a significant reduction in colony and spheroid forming ability in all investigated cell lines. Interestingly, a significant downregulation of epithelial marker E-cadherin and concomitant upregulation of mesenchymal markers such as vimentin and SNAIL was exclusively observed after TGF-β1 treatment. In addition, transcriptome profiling confirmed activation of gene sets involved in Cell Cycle:G1/S Checkpoint in response to both treatments (TGF-β1&β2) whereas enrichment in signaling pathways known to be involved in pro-metastatic properties resembling P13K, MAPK, MMPs and Hippo signaling pathway were predominantly associated with the TGF-β1 response.

Conclusions In conclusion, the cytostatic effect of TGF- β1 and TGF- β2 is reflected by a reduction in proliferation in both HCC and iCCA. Further, TFG-β1 seems to be an important regulator of EMT as well as invasive properties in progressed PLCs. Transcriptome profiling and Proteomics data indicates an increase in p21 that induces cell cycle arrest upon treatment of TGF-β1&2, while an increase in EMT related properties is associated only with the TGF-β1. These context-dependent dichotomic effects should be considered in TGF-β based therapeutic approaches.

4.32 Dimethyl fumarate (DMF) inhibits migration and proliferation of hepatocellular carcinoma (HCC)

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Introduction Dimethyl fumarate (DMF) is used to treat psoriasis (Fumaderm®) and multiple sclerosis (Tecfidera®). Recently, we have shown that DMF treatment leads to induction of cell death in NF-KB-dependent tumors. Cell death is induced by DMF-mediated formation of the ripoptosome which induces apoptosis and/or necreptosis. A mouse tumor model revealed that DMF application led to inhibition of metastasis formation, which was NF-KB-independent. Thus, we analyzed NF-KB-independent tumor cell lines (*e.g.* from colon, pancreas) in which DMF led to reduced migration and invasion. Recently, we analyzed whether DMF application leads to an inhibition of migration in NF-KB-independent HCC cell lines.

Methods Human HCC cell lines HepG2, Huh7 and Hep3B were treated with DMF (25 µM up to 100 µM) for up to 72 h. The cellular ATP content was measured using a luminescence based assay. To test the effects on migration a scratch assay with HCC cell lines was performed. DMF-treated cells were co-incubated with zVAD (50 µM) a caspase inhibitor to prevent induction of apoptosis. Cell migration was analyzed by microscopy. In addition, cell proliferation of HepG2 cells was determined. HepG2 were stained with Cytopainter Cell Proliferation Staining Reagent. The dye is divided between mother and daughter cell after each cell division. The proliferation of HepG2 was monitored after 24 h, to 96 h by FACS analysis.

Results DMF application resulted in a dose-dependent reduction of ATP-concentration in all cell lines. The strongest effects were observed upon 72 h treatment at a concentration of 100 µM DMF. Additionally, DMF application resulted in a dose dependent inhibition of migration of Huh7 and Hep3B cells. Migration of Hep3B cells was inhibited at a concentration of 25 µM DMF. A concentration of 100 µM DMF led to a complete block of migration in Hep3B

cells. Migration inhibition of Huh7 cells was less effective. In addition, we analyzed proliferation of HepG2 cells which was inhibited time- and dose-dependent by DMF application.

Conclusion Solid tumors (*e.g.* HCC) display a rather high mortality rate due to formation of metastasis. Here, we could show that DMF is capable to inhibit migration and proliferation in HCC cell lines. To identify the exact molecular mechanism is a challenging task for the future. Compared to anti-cancer drugs DMF shows less side effects, DMF is clinical approved and could be used as a basis to develop novel treatment options against metastasis formation.

4.33 Analysis of the expression and function of KIAA1199 in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is one of the most common cancers worldwide. Especially at advanced stages, therapeutic options are very limited. KIAA1199 is a protein that has been shown to participate in several cellular signaling pathways as well as cell proliferation. It has been described as tumor promoter but also as tumor suppressor depending on the type of cancer.

The aim of this project was to analyze the expression and function of KIAA1199 in HCC.

Methods and Results Human HCC cell lines (HepG2, Hep3B and PLC cells) showed reduced KIAA1199 protein expression compared to primary human hepatocytes. RT-qPCR analysis showed a downregulation of KIAA1199 in human HCC specimens compared to corresponding adjacent non-tumorous liver tissue. The observed downregulation was also confirmed by immunostaining of a tissue microarray consisting of paired tumor and non-tumorous liver tissues of HCC patients. As revealed by immunofluorescence, hepatic KIAA1199 is predominantly located in the cytoplasm of the HCC cells. Analysis of HCC patient data showed that KIAA1199 expression was lower in high risk patient group (based on prognostic index) and correlated inversely with patients' survival. Treatment with the HDAC inhibitor trichostatin A affected KIAA1199 expression in HCC cells, indicating epigenetic regulation. Interestingly, also treatment with sorafenib caused an upregulation of KIAA1199 expression in HCC cells. In order to assess the effect of KIAA1199 on HCC cell proliferation, its expression was suppressed applying specific siRNA in HCC cell lines. Subsequently, cell proliferation analyses as well as clonogenic assays were performed. Surprisingly, HCC cells transfected with KIAA1199 siRNA showed significantly decreased proliferation, which was accompanied by decreased CyclinD1 mRNA expression. Furthermore, KIAA1199-suppressed HCC cells revealed decreased colony formation ability compared to cells transfected with control siRNA.

Summary and conclusion KIAA1199 expression is downregulated in HCC, indicating an anti-tumorigenic role. In contrast to that, functional analyses in si-transfected HCC cells revealed a pro-proliferative effect of the protein. Thus, the role of KIAA1199 in HCC seems to be of complex nature, and requires further investigation.

4.34 Genetic fine analysis of the pro-fibrotic and pro-carcinogenic functions of Cyclin E1 in the liver

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Question Hepatocellular carcinoma (HCC) is one of the most severe tumor diseases with increasing incidence and limited treatment options. We have recently shown that the G1/S-phase cyclin, Cyclin E1 (CcnE1), is essential for the onset of liver fibrosis and the initiation of HCC. However, the precise contribution of CcnE1 in different cell compartments besides hepatocytes is incompletely understood. In the present study, we examined the role of CcnE1 in hepatic stellate cells (HSCs) for liver fibrogenesis, HCC initiation and progression.

Methods In order to delete CcnE1 specifically in HSCs we crossed conditional CcnE1 knockout mice ($CcnE1^{f/f}$) with transgenic mice expressing cre-recombinase under the control of the L-rat promoter resulting in mice lacking CcnE1 in HSCs ($CcnE1^{\Delta HSC}$). Induction of fibrosis and HCC was performed using the DEN/CCl_4 model. Briefly, mice were injected with a single dose of diethylnitrosamine at the age of 14 days followed by a weekly injection of carbon tetrachloride (CCl_4) beginning from the age of 6 weeks for up to 18 weeks. Mice were analyzed for liver fibrosis formation by histologic Sirius red staining. HCC initiation was determined by quantification of histologic pre-malignant lesions; as a measure of HCC progression, the number and size of macroscopic tumor nodules was evaluated.

Results Mice with deletion of CcnE1 exclusively in HSCs revealed significantly reduced liver fibrosis in comparison to cre-negative littermates after DEN/CCl_4 treatment. However, $CcnE1^{\Delta HSC}$ mice did surprisingly not show a reduced number or size of pre-malignant lesions in liver sections as initially hypothesized. In line, $CcnE1^{\Delta HSC}$ mice revealed a similar number and size of macroscopic tumor nodules.

Conclusions We provide evidence that HSCs are the major effector cells for the previously shown pro-fibrotic function of Cyclin E1. However, inhibition Cyclin E1 in HSCs is not sufficient to prevent onset of liver cancer suggesting that the postulated HCC-inducing impact of activated HSCs has been overestimated. We conclude that a theoretical anti-Cyclin E1 therapy of patients with liver fibrosis and high risk for HCC development could be beneficial provided that Cyclin E1 will be inhibited in most liver cells including HSCs and transformed hepatocytes. In summary, our study contributes to a better understanding of the complex, cell type-specific roles of CcnE1 for the pathological sequence from liver fibrosis to HCC.

4.35 Soluble urokinase plasminogen activator receptor (suPAR) predicts outcome after resection of biliary tract cancer

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Background Surgical resection is the only curatively intended therapy for patients with biliary tract cancer (BTC), but 5-year survival rates after tumor resection have remained below 30%, corroborating the need for better preoperative stratification tools to identify the ideal surgical candidates. The soluble urokinase plasminogen activator receptor (suPAR) represents a mediator of inflammation and has recently been associated with cancer. In this study, we evaluated a potential role of suPAR as a novel biomarker in patients undergoing resection of BTC.

Methods Tumor expression of uPAR, the membrane bound source of suPAR, was analyzed by IHC in 108 BTC samples. Serum levels of suPAR were analyzed by ELISA in a training and validation cohort comprising a total of 117 BTC patients and 76 healthy controls.

Results A high tumoral uPAR expression was associated with an adverse outcome after BTC resection. In line, circulating levels of suPAR were significantly elevated in BTC patients compared to healthy controls and patients with primary sclerosing cholangitis (PSC). Using a small training set, we established an optimal prognostic suPAR cut-off value of 3.72 ng/ml for BTC patients.

Importantly, preoperative suPAR serum levels above this cut-off value were associated with significantly impaired overall survival in both the training and validation cohort. Multivariate Cox-regression analysis including clinicopathological parameters such as the tumor stage, markers of systemic inflammation or organ dysfunction and established tumor markers revealed suPAR as an independent prognostic marker following BTC resection. Finally, high preoperative suPAR levels were indicative for acute kidney injury after tumor resection.

Conclusion Circulating suPAR represents a previously unrecognized biomarker in patients with resectable BTC, which might be useful to preoperatively identify the ideal candidates for tumor resection.

4.36 The relevance of mitochondrial BAX re-distribution for apoptotic evasion in human hepatocellular carcinoma

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Background Resistance to cell death is a hallmark of many cancers including hepatocellular carcinoma (HCC). The chronic inflammatory microenvironment within liver predisposes HCC development and progression. Herein, tumor predisposition to apoptosis is reflected by the dynamic distribution of the pro-apoptotic BCL-2 proteins BAX/BAK between cytosol and mitochondria. However, underlying molecular mechanisms that maintain this equilibrium during hepatocarcinogenesis remain elusive.

Methods We employed integrative functional and molecular analyses and revealed distinct tumor biology in a subgroup of HCC patients determined by a shift of mitochondrial Bax but not Bak pool to the cytosol during malignant transformation.

Results The HCC subgroup with predominant cytosolic BAX localization harbored selective protection from BAX activation and apoptotic cell death, respectively. Molecularly, this subgroup showed enrichment of signaling pathways associated with oxidative stress response and DNA repair as well as increased genetic heterogeneity. In contrast, non-protected HCCs followed activation of classical oncogenic networks. Importantly, gene expression profiles of protected HCCs were enriched in poorly differentiated HCCs and showed a significant association to the overall survival of HCC patients. Consistently, addiction to DNA repair of protected cancer cells resulted in profound apoptosis induction of cells otherwise insensitive to a variety of cell stresses upon PARP inhibition.

Conclusion Together, our results confirm that predisposition to mitochondrial apoptosis impairs tumor biology in HCC and might identify a subgroup of HCC patients with a specific response pattern and activated DNA repair mechanisms.

4.37 HER2 amplification is a rare event in non-liver-fluke associated cholangiocarcinogenesis

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Question Cholangiocarcinoma is a rapidly fatal cancer entity with a median survival of less than one year. In contrast to many other malignancies, no substantial therapeutic breakthrough has been made in the past decades limiting the treatment to cytotoxic chemotherapy with little beneficial effect for most patients. Targeted therapy tailored for the individual has shown substantial success in the recent past as a promising avenue for cancer therapy.

Methods In this study, we determined the frequency of gene amplification of HER2 in a comprehensive and well-characterized European cholangiocarcinoma cohort encompassing 436 patients with intrahepatic (n = 155), proximal (n = 155) or distal (n = 126) cholangiocarcinoma by strict application of a combined immunohistochemical and in situ hybridization algorithm following the current guidelines for HER2 assessment in gastric cancer.

Results We identified a proportion of 1.4% (n = 6) patients that demonstrated HER2 gene amplification, with the highest rate among the distal cholangiocarcinoma patients (2.4%). None of the patients with equivocal (2+) immunohistochemical staining results exhibited gene amplification molecularly. In four of five patients with HER2 positivity, gene amplification was already present in concomitantly tested high-grade biliary intraepithelial neoplasia (80%). HER2 gene amplification was not significantly associated with other clinical parameters, including survival.

Conclusions This study identifies HER2 gene amplification as a rare event in cholangiocarcinoma of the Western population, already occurring in high-grade BilIN in a subset of patients. Furthermore, we provide a robust testing algorithm that may be used prior to therapy administration in future phase II/III trials evaluating the role of HER2 as a predictive marker in cholangiocarcinoma.

4.38 Generation of syngeneic murine hepatoma cells bearing features of human HCC for rapid anti-HCC drug screening

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Background and Aims Hepatocellular carcinoma (HCC) represents the most frequent primary liver malignancy and is one of the leading causes of cancer related death worldwide [L.Kulik, Gastro. 2018]. HCC evolves in a long-term process with accumulation of (epi)genetic alterations that lead to the activation of oncogenic pathways. We aimed to derive hepatoma cells (HC) from a novel mouse model for HCC, reflecting chronic liver damage and fibrosis together with inherited and acquired genetic mutations to enable rapid anti-HCC in vivo drug screening.

Method and Results FVB Mdr2 knockout (KO) mice were injected with diethylnitrosamine (DEN, 10 µg/g body weight) at 3 weeks of age and exposed to phenobarbital (0.05%) in their drinking water. 8 months old HCC bearing mice were sacrificed and macroscopic lesions (measuring up to 5 mm) were carefully separated from non-tumorous tissue, minced and transferred into supplemented Dulbecco's modified Eagle's medium. After 8 months of subculturing, HC were harvested and transcript levels for HCC markers were quantified by qPCR. Compared to RNA extracted from livers of healthy FVB mice, the HC exhibited a high expression (> 100-fold) of alpha fetoprotein (AFP) and epithelial cell adhesion molecule (EpCAM), while transcript levels

of the HCC suppressor gene HNF4a were significantly downregulated. HE-staining revealed all signs for malignancy, such as a high nucleus/cytoplasm ratio with hypertrophy and a high number of mitoses with atypical mitotic forms. Downregulation of E-cadherin compared to control hepatocytes indicated epithelial-mesenchymal transition of the HC. In a first testing, Sorafenib attenuated HC proliferation in a dose-dependent manner with an IC 50 value of ~7 µM as demonstrated before for the HCC cell line HepG2.

Conclusion We have established a new HC line from our syngeneic Mdr2-/- HCC mouse model that replicates major features of human HCC. The cells display histologic signs of malignancy and characteristic HCC tumor markers as determined on the transcript and protein level. Sorafenib showed a dose-dependent antiproliferative effect on these HC. Conclusively, the HC are a promising cell model for ex vivo rapid screening of drug candidates, to be validated in the in vivo model and finally in patients.

4.39 Survival prediction for patients with ICC undergoing chemotherapy: comparing tumor markers to cross-sectional imaging

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Purpose Carbohydrate-antigen 19 – 9 (CA19 – 9) has been established as the main serum marker for patients with intrahepatic cholangiocarcinoma (ICC). The aim of this study was to compare the prognostic value of CA19 – 9 changes versus response determined by cross-sectional imaging in patients with ICC undergoing chemotherapy.

Methods and materials Between 2003 – 2018, 151 patients with histopathologically-confirmed ICC underwent chemotherapy at our tertiary care center for non-resectable or recurrent ICC, of whom 121 patients were included in this study. CA19 – 9 serum levels and imaging were retrospectively evaluated during chemotherapy. Log-rank testing and optimal stratification was used to classify patients into risk groups.

Results Prior to chemotherapy, baseline CA19 – 9 serum levels above the previously published cut-off of 37 U/ml were associated with poor survival (median OS 8.7 vs. 12.4 months, p = 0.003). After beginning of chemotherapy, an increase in CA19 – 9 of more than 40 U/ml resulted in impaired residual survival (median OS 5.0 vs. 12.1 months, p = 0.001). However, progressive disease at the first follow-up imaging proved the strongest predictor for poor outcome (median OS 4.6 vs. 15.5 months, p < 0.001).

Moreover, an increase of CA19 – 9 of more than 55 U/ml resulted in a positive predictive value for progressive disease in imaging of 76 %, whereas a decrease in CA19 – 9 of more than 2 U/ml resulted in a negative predictive value for progressive disease of 91 %.

Conclusion While an increase in CA19 – 9 serum levels during chemotherapy was an even better predictor than baseline CA19 – 9 levels for survival, response evaluation by cross-sectional imaging showed the best predictive performance towards residual OS in our cohort.

4.40 Real-life efficacy and safety profile of TACE in patients with intermediate HCC in a large German cohort

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Introduction Transarterial chemoembolization (TACE) is the standard of care for patients with locally advanced hepatocellular carcinoma (HCC). A recent meta-analysis demonstrated a median overall survival (OS) of 19.4 months and most common intervention-related adverse events included postembolization syndrome (PES)-associated symptoms in up to 47 %. The aim of this study was to validate the TACE intervention in terms of outcome and safety profile in large German cohort over a period of 10 years.

Methods and Patients Within this retrospective analysis we reviewed electronic charts of 987 patients diagnosed with HCC, who were treated between January 2008 and October 2017. Finally, 321 patients received TACE in palliative intention and were eligible for final analysis. Data of risk-factors, liver function, BCLC stage, ECOG, laboratory values, radiological findings, treatment modalities, and follow-up were recorded until 31.10.2017. Primary outcomes were median OS. Secondary endpoints were safety-profile of the intervention in terms of PES and median OS depending on Child-Pugh-status (CPS), ECOG, and BCLC-status.

Results The median OS in the complete cohort was 19.0 months. The median OS in patients classified Child A demonstrated a prolonged OS of 19.8 months compared to Child B with 8.6 months ($p < 0.0001$). OS-data in dependence of the ECOG at treatment-start were as follows: ECOG 0 22.3 months, ECOG 1 13.0 months, ECOG 2 9.2 months ($p < 0.0005$). Finally, we analyzed patients with respect to the BCLC-stage: BCLC B 16.8 months and BCLC C 8.7 months ($p < 0.002$). During 1064 TACE interventions 528 cases (49.6 %) of intervention-related adverse events occurred. The PES was present after 335 procedures (36.0 %) compared to 47.7 % annotated by the comprehensive meta-analysis by *Lencioni et al*. Of note and as expected, intervention-related adverse events occurred significantly more frequent after non-selective interventions (48.8 % vs. 40.1 %; $p < 0.05$).

Conclusion Our data demonstrated a comparable outcome compared to the most recent comprehensive literature. Notably, Child B and ECOG 2 patients were exposed to a substantial dismal prognosis, leading to critical discussion of TACE-indication and switching to alternative treatment options. The tolerability in terms of PES was markedly less common as indicated by the literature and non-selective TACE is significantly associated with higher rates of intervention-related adverse events.

4.41 Impact of complexity of laparoscopic liver resections on postoperative complications

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Background Operative time, conversion rate and blood loss were recently found to predict the difficulty of laparoscopic liver resections (LLR). Based on these three intraoperative variables a difficulty score for LLR was created. We aimed to assess if complexity of LLR also reflects postoperative complications.

Methods Patients who underwent LLR between November 2016 and August 2019 at the University Medical Center Freiburg were included. Resections were divided into three groups based on their complexity. Postoperative complications were defined according to Dindo-Clavien and the comprehensive complication index (CCI).

Results Of 76 patients, 75 %, 7 % and 18 % underwent low (group 1), intermediate (group 2) and high grade (group 3) difficulty of LLR, similarly distributed between patients with malignant and benign disease. Most frequently, complications occurred in group 3 (57 % vs. 20 % in group 2 and 19 % in group 1; p = 0.052), but major complications (p = 0.281) and mean CCI were similar between the different grades of complexity of LLR.

Conclusion Although difficulty of LLR reflects the occurrence of postoperative complications there was no association with their severity.

4.42 Induction of apoptosis in hepatocellular carcinoma by a novel combination of histone deacetylase inhibitor LBH589

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Background and Aims Hepatocellular carcinoma (HCC) is the most common primary tumor of the liver and the third leading cause of cancer deaths worldwide. HCC cells show a high apoptotic capacity in earlier stages of carcinogenesis, whereas in advanced stages they gradually develop apoptosis resistance. This anti-apoptotic phenotype is associated with development and progression of HCC. Due to its chemoresistance, novel treatment options for HCC are in urgent demand. Histone deacetylase inhibitors promote apoptosis via the intrinsic apoptotic pathway, but at the same time sensitize tumor cells to death ligands that activate the extrinsic apoptotic pathway. The chemotherapeutic drug bleomycin causes single- and double-strande DNA breaks and produces reactive oxidative species leading to cell death. The aim of this study is to identify novel drug combinations in order to induce cell death in HCC cell lines and thus to extend the therapeutic repertoire for HCC.

Method The human hepatoma cell line HepG2 was incubated with serum concentrations of histone deacetylase inhibitor LBH589 (0.1 – 5 µM) and bleomycin (2 – 10 µM). DMSO treatment served as control. Cell death was measured after 12 and 24 h via flow cytometry using DAPI/Annexin V-APC staining. The caspase Inhibitor Z-VAD-FMK and the RIPK1 inhibitor Nec-1 were added to investigate the effects of treatment combinations on cell death. Caspase cleavage as well as levels of pro-/anti-apoptotic members of the Bcl-2 family were determined by Western blot.

Results 24 h treatment with 0.1 µM LBH589 induced a strong increase in cell death of up to 6.5-fold compared to the controls. 2 µM bleomycin resulted in an increase of cell death of up to 4.5-fold. After incubation with a combination of LBH589 0.1 µM and bleomycin 2 µM cell death rates were increased to up to 14.8-fold, indicating a synergistic effect. Cell death could effectively be blocked by Z-VAD-FMK indicating induction of apoptosis. Incubation with LBH589, bleomycin and the combination of both therapeutics resulted in a cleavage of Caspases-3,-8,-9 and PARP, as well as in a downregulation of Bcl-XL as features of apoptosis.

Conclusion Our study demonstrates that novel combinations of already approved drugs like LBH589 and bleomycin could improve treatment options for HCC. Both drugs target different cellular pathways leading to synergistic effects and massive induction of apoptosis.

4.43 An umbrella concept for patients with advanced intrahepatic cholangiocarcinoma

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While the genomic diversity of biliary tract cancers and the relevance of its primary location have been recognized well, treatment of patients with advanced disease is mostly limited to conventional chemotherapy. However, recent clinical trials (NCT02989857) have shown success of a personalized treatment approach in advanced cholangiocarcinoma patients. Here, we present the umbrella concept of the Liver Cancer Center Heidelberg (LCCH) aiming at personalization of treatment, application of precision medicine, and enrichment of clinical studies.

Patients with advanced intrahepatic cholangiocarcinoma were screened for druggable targets in the LCCH since 2018. The Oncomine™ Comprehensive Assay v3 was used to interrogate genetic alterations in 161 genes. In addition, gene fusions were detected using the Archer Archer® Comprehensive Solid Tumor Assay.

Overall, 51 patients with intrahepatic cholangiocarcinoma were analyzed so far. 75% of detected potentially protumorigenic alterations were considered druggable. Based on the precision oncology concept of the HiGHmed consortium (use case oncology) and adopting the local study portfolio, likely druggable genetic alterations were identified in 77% of patients (range: 1 – 5 drug targets). Druggable gene fusions were observed in 10% of patients. A detailed overview and individual cases will be presented during the meeting.

Our umbrella concept identifies therapeutic targets in most patients suffering from advanced intrahepatic cholangiocarcinoma and thus warrants for a consistent application. The translation of this concept is hampered by the availability of safe drugs to be tested in suitable clinical trials, but may be supported by structural programs for personalized medicine currently developing in Germany (e. g., *Zentren für personalisierte Therapie*).

4.44 Multispectral Imaging to define morpho-molecular classes of human HCC

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Question Hepatocellular carcinoma (HCC) represents a molecularly and morphologically heterogeneous tumor entity. Molecular profiling allowed for molecular HCC classification, finally resulting in the definition of morpho-molecular subtypes by the new WHO classification. Although there is some evidence that immunohistology may allow for the identification of molecular HCC classes, a decisive protein-based HCC classification has not been performed yet and the complex interplay between pathways commonly altered in HCC as well as the proteomic heterogeneity of human HCC remain elusive.

Methods Formalin-fixed, paraffin-embedded (FFPE) tissues of 59 human HCCs were analyzed using multispectral Imaging (MSI, Vectra® 3.0 Automated Quantitative Pathology Imaging System), which enables the parallel detection of six biomarkers, while preserving the morphologic (image) information. Antibody panels were designed to interrogate molecular HCC subclasses (BrEP4, p-S6K, CRP, CTNNB1, GS, ARID 1A) as well as important HCC pathways (p-AKT, TP53, MDM 2, p-ATF2). As conventional image analysis software do not allow for the evaluation of cross-talks between signaling pathways, T-distributed Stochastic Neighbor Embedding (t-SNE) and machine learning based approaches were applied to analyze the multi-dimensional MSI data sets.

Results MSI-based classification of human HCC is feasible, but may be limited by background fluorescence. Importantly, both tumor and stromal cells contribute to molecular HCC signatures and MSI allows for the visualization of tumor heterogeneity as well as the interrogation of mechanism contributing to therapy resistance.

Conclusion Multiparameter immunohistology and advanced data analyses allow for morpho-molecular typing of HCC in routine FFPE biopsies, thereby demonstrating its potential power to identify predictive biomarkers highlighting the importance of HCC biopsy in clinical trials.

4.46 LINC00152 drives a ceRNA network in human hepatocellular carcinoma

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Increasing evidences have assigned essential roles in cellular processes to the long non-coding RNAs (lncRNAs). These include chromatin organization, gene transcription, RNA turnover and maturation. More interestingly, lncRNAs may act as miRNA sponges thereby affecting miRNA bioavailability, which in turn alters target gene expression. By integrating methylome and gene expression data of human hepatocellular carcinoma (HCC) samples we observed methylation-dependent downregulation of LINC00152 in human HCCs compared to normal liver. Here, we investigated whether LINC00152 forms a competing endogenous RNA (ceRNA) network in human HCC.

Using *in silico* analyses (http://www.mircode.org) 24 miRNA candidates were predicted to bind to LINC00152. Of these, expression of 22 miRNAs was detected in a cohort of human HCC samples. Next, the genes potentially regulated by these miRNA candidates were determined using StarBase. Based on the criterion to be predicted by at the least two different algorithms (http://targetscan.org, http://pictar.mdcberlin.de, http://www.microrna.org, https://cm.jefferson.edu/rna22v2/) 2.664 genes were identified as potential target genes potentially regulated by a LINC00152-driven ceRNA network. Based on the statistical association between putative miRNAs and target genes in our human HCC, miR.23a.3p, miR.125a.5p, miR.125b.5p, miR.223.3p, and miR.143.3p were selected as top miRNA and STK39, FAM60A, FUT4, PALLD, and MAP3K1 as top gene candidates. In line, decreased expression of these target genes was detected in LINC00152-deficient HuH7 cells compared to controls. More interestingly, RNA immunoprecipitation revealed that LINC00152 co-occurs with all top 10 candidate miRNAs in ribonucleoprotein complexes (miRNPs). Inhibition or overexpression of LINC00152 in human HCC cell lines decreased or increased respectively cell growth compared to the corresponding control cells. Furthermore, high LINC00152 expression level was associated with shorter survival of HCC patients after liver resection.

All together our data demonstrate that LINC00152 drives human hepatocarcinogenesis via a ceRNA network.

4.47 Retrospective evaluation of RFA and MWA for the treatment of HCC at the University Medical Center Hamburg 2008 – 2016

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Purpose Ablation procedures are a recommended treatment option for patients with early hepatocellular carcinoma (HCC), who are not suitable for resection. The aim of this study was to compare the outcome and safety profile in a large cohort of patients with HCC receiving microwave (MWA) or radiofrequency ablation (RFA) at the University Medical Center Hamburg-Eppendorf.

Materials and Methods In this retrospective longitudinal single-center study we screened 987 patient records, who were diagnosed with HCC between January 2008 and December 2016 at our institution. Data on initial diagnosis, etiology of liver disease, liver function, BCLC stage, laboratory values, radiological response were recorded. Final data cut was 31.12.2017.

Results The overall cohort included 987 patients (800 men and 187 women) with a median age of 65 years (18 – 90 years). 91% presented with underlying liver cirrhosis at the time of HCC diagnosis. The main risk factors were: 37.6% alcohol-associated, 21.1% and 13.2% chronic hepatitis C or B, respectively, representing a typical western patient cohort. For our final analysis we were able to include 63 patients, who received an ablation treatment; 34 patients were treated with RFA (75 procedures) and 29 with MWA (76 procedures). The median OS was 27 months in the MWA group and 32 months in the RFA group ($p = 0.98$). Median recurrence-free survival (RFS) was 20 months in the MWA cohort and 21 months in the RFA cohort ($p = 0.34$). In the MWA group, a total of ten (13.2%) complications (CTCAE grade 1 or 2) occurred: 2 subcapsular hematomas, 2 events of abdominal pain, 5 superficial bleedings and 2 infections. Nine (12.0%) complications occurred in the RFA cohort: 1 patient died due to arterial bleeding, 1 major bile leakage and subcapsular hematoma, 5 events of diffuse abdominal pain, and 2 superficial bleedings.

Conclusion Our analysis demonstrated similar efficacy of both treatment modalities with comparable OS and RFS. Additionally, the safety profile was also comparable, mainly short-term abdominal pain, however one patient died due to an arterial bleeding in the RFA group.

4.48 Deletion of Sirtuin 6 affects tumor-directed innate immune responses in a syngeneic mouse model of hepatocellular carcinoma

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Advancing age is the most important risk factor for developing cancer. This occurs coincident to a decline in immune system function, termed immunosenescence suggesting a causal role for cancer development. Epigenetic mechanisms linked to ageing can contribute to cellular ageing and the discovery of ageing genes forms the molecular basis for this relationship. One of these genes is Sirtuin 6 (Sirt6), which belongs to the sirtuin family of NAD+-dependent deacetylases. Loss of Sirt6 in mice leads to a severe premature aging-like phenotype leading to an early death of the mice at the age of 4 weeks. Sirt6 is involved in epigenetic gene silencing through histone deacetylation. In our previous work we found a deregulation of differentiation-relevant genes in the livers of Sirt6 deficient mice resulting in an oncofetal phenotype. We could show that this gene signature is predictive for HCC patients with respect to survival and tumor recurrence rates.

In recent investigations we have analyzed the immune cell populations present in the bone marrow, blood and spleen and found aging-associated inflammation (inflammaging) like phenotype marked by increased number of myeloid cells in Sirt6-deficient mice. To analyze the role of Sirt6 in myeloid derived cells in antitumor immune responses we have generated a myeloid-specific Sirt6 knockout mouse model in which syngeneic hepatoma cells were injected. Analysis of this tumor model and comparative experiments on human cells indicates a role of Sirt6 in myeloid differentiation and function.

Thieme

Poster Visit Session V Viral Hepatitis and Immunology
Saturday, February 15, 2020, 11:00 am – 11:45 am, Lecture Hall P1

5.4 Aberrant expression of activation regulators *CBL-B*, *CTLA-4* and *PD-1* in intrahepatic T_{eff} cells in autoimmune hepatitis

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Question Autoimmune hepatitis (AIH) is a chronic inflammatory liver disease with unknown pathogenesis. Activated T effector cells seem to play a crucial role. Here, we tested the hypothesis that T cell activation in AIH is inappropriately controlled. We investigated various molecules involved in the regulation of T cell activation in liver and blood of patients with AIH in comparison to healthy individuals and to other immune-mediated liver diseases.

Methods A total of 86 AIH patients were examined. Gene expression levels of activation regulators *CBL-B, CTLA-4, GRAIL, ICOS, ITCH, NEDD4, OX40, PD-1, PKC ((Theta))* and *TRAF6* were analysed by qPCR of liver samples or isolated peripheral T cells of AIH patients and control subjects. RNA in-situ hybridization with selected probes and CD3 co-staining was performed on liver samples of AIH or DILI patients. The modified hepatic activity index (mHAI) score was applied to liver histologies to quantify intrahepatic inflammatory activity. Protein expression of intrahepatic T effector cells was examined by flow cytometry.

Results qPCR screening revealed that in livers of treatment-naïve AIH patients as compared to healthy control subjects, *CBL-B* ($p < 0.01$), *PD-1* ($p < 0.01$) and *CTLA-4* ($p < 0.001$) were significantly elevated. Intrahepatic expression of *CBL-B, PD-1* and *CTLA-4* in AIH patients correlated positively with the mHAI. RNA in-situ hybridization revealed that T cells positive for *CBL-B* ($p < 0.01$), *CTLA-4* ($p < 0.01$) and *PD-1* ($p < 0.02$) were increased in hepatic portal areas of treatment-naïve AIH patients as compared to DILI patients and to AIH patients under treatment. Flow cytometry revealed that levels of intrahepatic *CBL-B+CD4+*T cells ($p < 0.005$) and *CBL-B+CD8+* T cells ($p < 0.005$) were elevated in treatment-naïve AIH patients as compared to healthy controls. *CTLA-4+CD4+*T cells ($p < 0.005$), *CTLA-4+CD8+* T cells and *PD-1+CD8+* T cells were increased in treatment-naïve AIH patients in comparison to healthy controls.

Conclusion Intrahepatic T cells in active AIH maintain high levels of the activation inhibitor *CBL-B* following activation, which contrasts with previous studies in mice and in patients with multiple sclerosis. *CBL-B* expression correlated positively with histological activity of AIH. Moreover, the *CBL-B* modulators *CTLA-4* and *PD-1* were also up-regulated in intrahepatic T cells in active AIH. These findings indicate that intrahepatic T cell activation regulators are aberrantly expressed in AIH, thus contributing to its pathogenesis.

5.5 Autoaggression of $FOXO1^{low}CXCR6^{hi}CD8^+$ T cells causing liver pathology in NASH

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Question During chronic liver inflammation an imbalanced immune system causes liver damage. For chronic infectious inflammation it is known that the adaptive immunity drives liver pathology. But how liver damage is induced during chronic sterile inflammation like non-alcoholic steatohepatitis (NASH) is completely unknown. Appropriate integration of extracellular factors into a transcriptional network in $CD8^+$ T cells is indispensable to control effector function avoiding immune pathology. In the liver $CXCR6^+CD8^+$ T cells express high levels of effector molecules like GzmB and are important for tissue homeostasis. Here we identify FOXO1-activity in $CXCR6^+CD8^+$ T cells as critical regulator of CXCR6 expression and effector function during chronic sterile inflammation in NASH.

Methods RNA-seq, flow cytometry and cytotoxicity assays were performed to study immunity of $CXCR6^+CD8^+$ T cells and its dependence on FOXO1 *in vitro* and *ex vivo* studies. Murine model of non-alcoholic steatohepatitis (NASH) was used to explore FOXO1-dependent T cell immunopathology.

Results RNA-seq analysis of $CXCR6^+CD8^+$ T cells of the liver from NASH mice compared to $CXCR6^+CD8^+$ T cells from WT mice revealed strong upregulation of effector and inhibitory molecules with FOXO1 as the central transcription factor. Flow cytometric analysis confirms a $FOXO1^{low}CXCR6^+GzmB^{high}CD69^+PD1^+CD8^+$ T cell population that was highly and specifically increased in the liver of NASH mice. We discovered IL-15 and IL-21 as critical cytokines downregulating FOXO1 accompanied with an increase of $CXCR6^+CD8^+$ T cells. Treatment of NASH mice with an anti-CD122 antibody abrogated the generation of $CXCR6^+GzmB^{high}CD8^+$ T cells associated with a strong amelioration of liver disease. Mechanistically, hepatocytes were killed *in vitro* by $CXCR6^+CD8^+$ T cells with lower FOXO1 activity through an MHC class I independent but ICAM-LFA1 dependent manner. Inflammatory levels of TNFa were critical to upregulate ICAM and to facilitate formation of an immunological synapse of hepatocytes with $CXCR6^+CD8^+$ T cells even in the absence of antigens. Since ICAM cluster were higher in NASH mice, we assume that increased effector function in $CXCR6^+$ $CD8^+$ T cells in the absence of FOXO1-control caused antigen-independent hepatic immunopathology.

Conclusion Our results provide evidence for a critical role of FOXO1 activity in $CXCR6^+CD8^+$ T cells of the liver controlling effector function that is required to prevent liver immunopathology.

5.6 *Il-2*, *Il-22* and *Rantes* upregulation in a new mouse model of bacterial infection related acute-on-chronic liver injury

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Background and aims Bacterial infection (BI) is a common precipitant of acute-on-chronic liver failure (ACLF) in humans. Therefore, we aimed to establish a novel BI-related ACLF (BI-ACLF) model by intraperitoneal (IP) lipopolysaccharide (LPS) injection to a knock-out mouse with a pre-existing chronic liver injury (*Abcb4-/-*).

Methods Fifteen week-old C57BL/6J ($N = 16$) (wild type, wt) or *Abcb4-/-* ($N = 16$) (knock-out, ko) mice were treated with IP injections of either LPS (4 mg/kg) or saline solution (0.9% NaCl). Six hours post-injection, plasma/liver samples were collected. Hepatic expressions (relative to *Gapdh*) of interleukin-6 (*Il-6*), C-reactive protein (*Crp*), tumor necrosis factor-α (*Tnf-α*), regulated on activation, normal T cell expressed and secreted (*Rantes*), Toll like receptor 4 (*Tlr4*), monocyte chemoattractant protein-1 (*Mcp1*), interleukin 10 (*Il-10*) in-

terleukin-2 (*Il-2*) and interleukin-22 (*Il-22*) were evaluated by 2-ΔΔCt method. Paired t-test was used for statistical evaluation and $p < 0.05$ was considered as significant.

Results LPS challenge resulted in dramatic elevation of hepatic *Mcp1*, *Rantes*, *Il-6, il-10* and *Tnf-α* expression with approximately 200-, 60-, 54-, 52- and 40-fold increases in wt mice, respectively. A considerably moderate upregulation in *Crp* (4-fold), *il-2* (6-fold) and *Tlr4* (10-fold) was also observed in livers of wt mice after exposure to LPS. Corresponding effects were also found in *Abcb4-/-* mice with no significant differences in terms of liver specific expressions of *Il-6*, *il-10*, *Tnf-α*, *Tlr4*, *Mcp1* and *Crp* compared to wt mice. On the other hand, LPS resulted in a more profound upregulation of *Rantes* (135-fold vs. 60-fold increase, $p < 0.001$) and *Il-2* (41-fold vs. 6-fold increase, $p < 0.001$) expressions in *Abcb4-/-* mice in comparison to wt mice. No hepatic *Il-22* mRNA was detected in NaCl-administered mice, while LPS stimulated hepatic *Il-22* expression in both genotypes with a 7.3-fold increase in ko mice compared to wt counterparts ($p < 0.001$).

Conclusions We propose a novel promising approach to model BI-ACLI in *Abcb4-/-* mice with high expression levels of hepatic cytokines and chemokines after LPS challenge. While excessive inflammatory responses might be dampened in this dual hit model after acute LPS insult, *Il-2*, *Il-22* and *Rantes* are evidenced to specifically trigger inflammatory cascades with harmful consequences on further disease progression in this model.

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5.7 HCV-specific CD4 T cells show strong Tfh functionality after DAA-mediated viral clearance

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Background During Hepatitis C virus (HCV) infection peripheral HCV-specific CD4 T cells acquire a T follicular helper (Tfh) phenotype but rapidly disappear from circulation and seem to accumulate in the liver tissue as the infection progresses to chronicity. Recently we revealed that HCV-specific Tfh cells with a memory-like phenotype reappear in the periphery after direct acting antiviral (DAA)-mediated viral clearance but their low frequency prevented us from further functional research on these cells.

Methods We established a clone-based system to evaluate CD4 T cell functionality *in vitro*. CD4 T cell clones were generated by single cell sorting and antigen non-specific expansion. Influenza- (Flu-) and HCV-specific CD4 T cell clones were established from healthy donors (HD) and DAA-cured HCV patients, respectively. Tfh (CXCR5+, PD-1+, CXCR3-) and Th1 (CXCR5-, CXCR3+, CCR6-) clones with unknown antigen-specificity derived from HD or patients. Phenotype and cytokine production were analyzed by flow cytometry. The B cell helper capacity was assessed by coculture of naïve allogeneic B cells with the T cell clones.

Results Differences in phenotype and functions of Tfh and Th1 clones demonstrated the maintenance of lineage-specific features *in vitro* and therefore validated our methodological approach. Unlike Tfh, Th1 and Flu clones, HCV clones showed less alteration of their *ex vivo* phenotype maintaining high PD-1 and CXCR3 expression. Furthermore, HCV clones were highly activated, indicated by high CD38 and OX40 expression but also characterized by elevated levels of the inhibitory markers TIGIT and BTLA. Despite their unique phenotype that was clearly different from Tfh clones, HCV clones showed high B cell helper capacity. Moreover, HCV clones produced high amounts of TNF, IL-21 and IFNg with a cytokine profile that more closely resembled that of Tfh clones.

Conclusion The highly functional potential of HCV-specific CD4 T cells after DAA-mediated elimination of persistent infection suggests that chronic antigen and interferon exposure of intrahepatic HCV-specific CD4 T cells *in vivo* might not have resulted in functional exhaustion but rather altered Tfh functionality to facilitate long-term viral control. Displaying such strong Tfh characteristics HCV-specific CD4 T cells after DAA therapy could be targeted by vaccination as they would support B cell-mediated humoral immunity.

5.8 Adverse effects of PD-1 targeted immunotherapy in NAFLD-triggered HCC

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Immunotherapy has opened hitherto unknown possibilities to treat cancer. Whereas some cancer types (e.g. melanoma) are efficiently treated others lack measurable positive effects (e.g. PDAC). Hepatocellular carcinoma (HCC) has a dismal prognosis, limited treatment options and survival benefit, making it a target candidate for immunotherapy. Here we investigated NAFLD-triggered HCC in the context of a metabolic syndrome and PD1-targeted immunotherapy. Using flow cytometry, single cell Seq and proteome analysis, we found a progressive increase of CD8+ effector T-cells with distinct exhaustion profiles concomitantly rising with NASH severity. We found that PD-1-targeted immunotherapy had a dismal treatment outcome at the time point of HCC initiation or at late stage HCC. We identified pre-dysfunctional PD-1+CD8+ T-cells as main drivers of disease and hepatocarcinogenesis upon PD-1-targeted immunotherapy in NASH. Similar, in a study across 6 centers in Austria and Germany, patients with NAFLD/NASH-driven HCC under PD-1-targeted immunotherapy had reduced time to progression and progression-free survival, translating to a significant worse overall survival compared to HBV, HCV or ASH-triggered HCC.

Thus, our data data indicate that PD-1-targeted immunotherapy induces adverse effects in NAFLD-driven HCC through activation of CD8+PD1+ effector T cells and NAFLD/HCC patients need to be stratified in more detail as potential non-responders with adverse effects in the context of immunotherapy.

5.9 Systemic CD4 T cell tolerance induction in the liver depends on interferon-γ and CTLA-4

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Introduction/Objectives We have previously shown that ectopic expression of myelin basic protein (MBP) in the liver can prevent autoimmune encephalomyelitis (EAE); however, the mechanisms explaining how the liver can effectively protect distant tissues are not entirely clear.

Methods MBP-specific tg4 T cells were adoptively transferred into CRP-MBP mice expressing MBP in the liver. Molecules associated with hepatic tolerance induction were identified by immunophenotyping of tg4 T cells retained from

the liver. Inhibitory antibodies were used to test the importance of the identified molecules for the protection from autoimmune disease in CRP-MBP mice.
Results Transferred tg4 T cells accumulated in the livers of CRP-MBP recipients, as compared to non-transgenic littermates (> 10-fold). This accumulation was facilitated by hepatic re-stimulation of tg4 T cells, resulting in increased IFN-γ production, and subsequent activation of the CXCL9 – CXCR3 axis, which enables endothelial transmigration. The accumulated tg4 T cells in the livers of CRP-MBP mice showed a significant up-regulation of several co-inhibitory receptors together with IFN-γ, including Lag-3, PD-1, Tim-3, TIGIT and, most notably, CTLA-4. However, blockade of CTLA-4 by in vivo administration of an anti-CTLA-4 antibody did not impair tolerance. In contrast, blockade of IFN-γ reduced expression of CXCR3 and CXCL9 and prevented hepatic accumulation of tg4 T cells, resulting in partial impairment of systemic tolerance and development of mild EAE. Intriguingly, concomitant blockade of IFN-γ and CTLA-4 completely abolished tolerance to MBP and induced severe EAE.
Conclusion Our findings demonstrate that systemic CD4 T cell tolerance induction in the liver and protection from autoimmune disease depends on 1) IFN-γ-mediated transmigration of circulating autoreactive T cells into the liver parenchyma and 2) up-regulation of multiple co-inhibitory receptors, notably CTLA-4.

5.10 Single cell RNA sequencing reveals naïve T cells ready for effector function in livers of Primary Sclerosing Cholangitis

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Question Although genetic associations point towards immune dysregulation as part of the pathogenesis of Primary Sclerosing Cholangitis (PSC), the role of specific immune cell populations within the liver of patients with PSC remains elusive. Here, we report on a comprehensive phenotypic and functional investigation of the liver infiltrating T cells of patients with PSC.
Methods Liver infiltrating and circulatory T cells were isolated from patients with PSC (n = 16). As controls we used T cells isolated from liver tissue and blood from patients undergoing transplantation for alcoholic liver disease (ALD, n = 16) and from blood of healthy donors (n = 10). Liver infiltrating T cells were sorted and processed for single cell RNA sequencing (scRNA-Seq) and cellular indexing of transcriptome and epitopes by sequencing (CITE-Seq). T cells were stained for measurements of surface markers by flow cytometry. Finally, naïve T cells were analyzed for proliferation capacity, cytokine production and differentiation capacity *in vitro*.
Results By combining scRNA-Seq, CITE-Seq and multi-parameter flow cytometry, we observed a unique immunophenotype of liver infiltrating and peripheral T cells in PSC, when compared to ALD and healthy controls. A subset of liver infiltrating naïve-like CD4+ T cells was identified in PSC patients with end-stage liver disease. Using flow cytometry, we confirmed the presence of phenotypically naïve CD4+ T cells in PSC livers and found they were increased in frequency compared with ALD. Transcriptome analysis using scRNA-Seq revealed high expression of markers such as *STAT3* suggesting a possible predisposition to acquire a Th17 phenotype. *In vitro*, PSC derived naive CD4+ T cells showed higher rates of proliferation and production of cytokines associated with Th17 cells compared to healthy controls.
Conclusion We here provide the first comprehensive atlas of intrahepatic T cells in PSC. Naïve-like CD4+ T cells ready to differentiate into Th17 cells were identified within the liver and their abundance was increased in liver and blood of PSC compared to ALD. We propose these cells as potential contributors to disease pathogenesis.

5.11 Cytokine patterns of newly identified HEV-specific CD4 T cell epitope

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Background Although immunocompetent individuals are able to spontaneously clear Hepatitis E Virus (HEV) infection, it is now well accepted that the emerging antibody response does not necessarily protect from re-infection, suggesting a lack of long-lasting neutralizing antibody responses. However, the successful vaccination against genotype 1 in China demonstrates the possibility for long-term protection. Furthermore HEV causes chronic infection and cirrhosis in immunocompromised hosts. In our study we aim to elucidate how CD4 T cells, especially follicular T helper (Tfh) cells, are involved in protection from HEV, as this subset is specialized to support B cells to mount a long-lasting and high affinity antibody response.
Methods Therefore, HEV-specific CD4 T cell epitopes (genotype 3 as most common genotype in Europe) were predicted *in silico* using the IEDB analysis resource consensus tool. Cytokine production of HEV-specific CD4 T cells was analyzed by flow cytometry after antigen-specific expansion of PBMCs from 26 patients and healthy donors (HD) that resolved HEV (rHEV) infection. Neutralizing antibody titers are currently being analyzed.
Results We identified 21 novel MHC class II restricted HEV-specific CD4 T cell epitopes in 25 out of the 26 rHEV patients/HD. Furthermore, we confirmed 5 of 6 previously described epitopes. More importantly, the individual epitopes induced different cytokine patterns. Whereas for some peptides a strong induction of IFN-g, IL-2 and IL-21 was observed, others failed to induce IL-21 production demonstrating a diversification of CD4 T cell response depending on the epitope with some epitopes predominantly inducing a Tfh response. Moreover, broader and stronger CD4 T cell responses were observed in patients that resolved HEV infection within the last 6 month pointing to a time dependence of a sufficient response to HEV.
Discussion Our results give first insights into the role of HEV-specific CD4 T cells during and after HEV infection. To further investigate the determinants of protection we will correlate the neutralizing antibody response to identify correlations between the breadth and quality of the Tfh cell response and the neutralizing antibody titers.

5.12 Oxygenation and miR138-5 p act as rheostats in APOBEC 3B-mediated Hepatitis B Virus control

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Hepatitis B virus (HBV) remains a major health problem with 257 million people chronically infected. Current treatments can control the infection but does not allow its complete eradication leading to relapses upon withdrawn or resistance development. These relapses are due to the persistence of the covalently closed circular DNA (cccDNA), which is not efficiently targeted by those treatments. Agonization of the lymphotoxin β receptor (LTβR) with BS 1 was shown to induce APOBEC 3B (A3B), which induces DNA damage into cccDNA, which eventually leads to its degradation (Lucifora *et al.*, 2014). Here, we analysed the underlying regulation mechanisms of A3B in the context of HBV-infection.

We show that A3B induction depends on NF-κB pathway signalling, as NIK knock-down and IKKβ inhibition, strongly impair the induction of A3B, reverting the antiviral effect of LTβR-activation. In addition, a latency in A3B induction is observed between 36 h to 72 h of BS 1 treatment, before a strong induction after 4 days onwards. We identified the micro RNA 138 – 5 p (miR-138-5 p) to be implicated in the regulation of A3B. Transfection of dHepaRG cells with the miR-138-p prevents the induction of A3B by BS 1, and HBV inhibition. Interestingly, even without the presence of BS 1, miR-138-5 p transduction increases HBV replication, highlighting a basal expression of A3B in HBV infected cells. Finally, in livers of HBV infected patients, hypoxic areas are associated with an increased level of HBV- replication and a decreased expression of A3B. *In vitro*, A3B expression is reduced during hypoxia through a HIF1α dependant mechanism. This leads to impaired A3B dependent cccDNA purging under hypoxia upon BS 1 treatment. Removing HIF1a from the system allows again A3B upregulation and antiviral effects.

Altogether, we have characterised the mechanisms of APOBEC 3B regulation in hepatocytes through NF-κB and miR-138-5 p. We have also highlighted a fine regulation between HBV infection, APOBEC 3B expression and hypoxia, explaining the persistence of HBV even during inflammation and LTβR-stimulation.

5.13 Development of liver-resident CD 8 T cells during persistent viral liver infection results in functional adaptation

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Question Tissue-resident CD 8 memory T cells (T_{RM}) are described to populate parenchymal organs and to be essential for an effective immune response against pathogen re-challenge. However, the role of T_{RM} cells in viral infections of the liver, like chronic Hepatitis B Virus (HBV) infection, is largely unknown. We aimed at monitoring and characterizing the formation of liver-resident T cell populations upon acute-resolving or chronic viral infection of the liver for OVA-based infection models as well as chronic Adeno-HBV infection in mice.

Methods C 57BL/6 mice were infected with liver-targeting adenoviral vectors encoding the model antigen ovalbumine under the CMV-, or the hepatocyte-restricted TTR-promoter to achieve acute-resolving or chronic viral infection, respectively. At different time points, virus-specific T cells from spleen and liver were analysed with respect to phenotypic and functional parameters. Similarly, mice were infected with a chronic dose of Adeno-HBV after having received naïve HBVcore-antigen-specific CD 8 T cells and analysed accordingly.

Results Upon resolved infection, remaining virus-specific CD 8 T cells in the liver subdivided into a CX_3CR1^+ effector-memory and a $CXCR6^+$ $CD69^+$ liver-resident memory population that could be clearly distinguished by phenotypic and functional analysis. In contrast, during chronic liver infection, virus-specific T cells were expanded and maintained exclusively in the liver. These cells exhibited a $CXCR6^+$ $CD69^+$ T_{RM}-like phenotype along with a functional adaptation characterized by low Granzyme B levels, inability to secrete cytokines and high expression of inhibitory receptors. Similar results were obtained for persistent Adeno-HBV infection where we confirmed the establishment of a virus-specific CD 8 T cell population in the liver that shares T_{RM}-characteristics, but exhibits functional impairment.

Conclusion Our results suggest that the development of $CXCR6_+$ liver-resident T cells occurs not only in the course of resolving, but also during chronic viral infection of the liver. In a chronic setting, however, $CXCR6^+$ cells do not represent a functional T_{RM} population, but exhibit attenuated effector functions that presumably reflect adaptation to the persistent viral infection. Ongoing work is dedicated to define the mechanisms of T_{RM} development during chronic infection and to find ways for reverting T cell attenuation through adequate stimulation.

5.14 A dual role for hepatocyte-intrinsic canonical NF-κB signaling in virus control

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Background & Aims Hepatic innate immune control of viral infections has largely been attributed to Kupffer cells, the liver macrophages. However, also hepatocytes, the parenchymal cells of the liver, possess potent immunological functions in addition to their known metabolic functions. Owing to their abundance in the liver and known functions, we aimed to investigate the direct anti-viral mechanisms employed by hepatocytes.

Methods Using lymphocytic choriomeningitis virus (LCMV) as a model of liver infection, we first assessed the role of myeloid cells by depletion prior to

infection. We investigated the role of hepatocyte-intrinsic innate immune signaling by infecting mice lacking canonical NF-κB signaling (IKKβ$^{\Delta Hep}$) specifically in hepatocytes. In addition, mice lacking hepatocyte-specific interferon-α/β signaling-(IFNAR$^{\Delta Hep}$), or interferon-α/β signaling in myeloid cells-(IFNAR$^{\Delta Myel}$) were infected with LCMV.

Results Here, we demonstrate that LCMV activates NF-κB signaling in hepatocytes. LCMV-triggered NF-κB activation in hepatocytes did not depend on Kupffer cells or TNFR1 signaling. LCMV-infected IKKβ$^{\Delta Hep}$ livers displayed strongly elevated viral titers due to LCMV accumulation within hepatocytes, reduced interferon-stimulated gene (ISG) expression, delayed intrahepatic immune cell influx and delayed intrahepatic LCMV-specific CD8+ T-cell responses. Notably, viral clearance and ISG expression were also reduced in LCMV-infected primary hepatocytes lacking IKKβ, demonstrating a hepatocyte-intrinsic effect. Similar to livers of IKKβ$^{\Delta Hep}$ mice, enhanced hepatocytic LCMV accumulation was observed in livers of IFNAR$^{\Delta Hep}$, whereas IFNAR$^{\Delta Myel}$ mice were able to control LCMV-infection. Hepatocytic NF-κB signaling was also required for efficient ISG induction and interferonα/β-mediated inhibition of HBV replication *in vitro,* in HBV- or HDV-infected HepaRGs.

Conclusions Together, these data show that hepatocyte-intrinsic NF-κB is a vital amplifier of interferonα/β signaling pivotal for early, strong ISG responses, influx of immune cells and hepatic viral clearance.

5.15 Both interferon and siRNA treatments promote reappearance of SMC5/6 complex but have different impact on cccDNA

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Treatment with siRNA targeting HBV transcripts is currently under investigation as a novel anti-HBV treatment approach. By reducing all viral transcripts, siRNA has the potential to reduce viremia and antigenemia but has no direct effect on cccDNA stability.

Aim of this study was to investigate these antiviral effects in HBV-infected human hepatocytes in vivo and its consequences on the "structural maintenance of chromosomes" complex SMC5/6, a host restriction factor able to repress cccDNA transcription but counteracted by HBx.

Methods & experimental design We treated HBV-infected humanized mice for 4 to 6 weeks with siRNA targeting all viral transcripts or with pegylated interferon alpha (peg-IFNα), or mice were left untreated. Viral parameters in the serum and liver, and interferon-stimulated genes (ISG), were assessed by qPCR. cccDNA levels were also analyzed by Southern blotting. RNA in situ hybridization coupled to immunofluorescence (RNA-ISH+IF) was used to visualize both HBV transcription and SMC6.

Results Both treatments reduced viral RNAs and proteins, including HBx, which targets SMC5/6 for degradation, thus promoting reappearance and epigenetic binding of SMC5/6 to the cccDNA. However, only interferon treatment reduced cccDNA levels. As expected, ISG expression was elevated by IFNα but not upon siRNA treatment. SMC6 protein was degraded in the livers of untreated HBV-infected mice, but was clearly detectable in mice treated with either IFNα or siRNA. After stopping IFN, entry inhibition strategies showed that HBV rebound mostly relied on new infection events.

Conclusions Our study shows that both IFNα and siRNA treatment is accompanied by the reappearance of the SMC5/6 complex in human hepatocytes in vivo. This indicates that not the induction of ISGs but the suppression/reduction of HBV RNAs and related protein production is sufficient to promote SMC5/6 rebound. Although HBV RNA reduction rescued SMC5/6, targeting cccDNA stability together with entry inhibition seem necessary to cure infected cells.

5.16 Inflammatory peritoneal MAIT cells accumulate during the early phase of spontaneous bacterial peritonitis

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Questions Mucosal-associated invariant T (MAIT) cells, which play important roles in anti-bacterial defence, are depleted from blood in advanced liver disease and show features of immune dysfunction. As circulating MAIT cells may differ from organ-resident MAIT cells, we aimed to investigate the frequency, phenotype, and function of peritoneal MAIT cells from patients with cirrhosis in the absence or presence of spontaneous bacterial peritonitis (SBP).

Methods MAIT cells were isolated from peripheral blood and ascitic fluid from 100 patients with decompensated cirrhosis using flow cytometry. Whole blood samples from healthy individuals and peritoneal dialysate from non-cirrhotic individuals served as controls. Co-culture experiments were performed to assess the response to PMA/Ionomycin, bacterial culture supernatants, and infected ascitic fluid. The migration of MAIT cells was studied using trans-well migration assays.

Results Peritoneal MAIT cells had an inflammatory, tissue retention phenotype expressing the integrins αEβ7 and α4β7, and the chemokine receptors CXCR3, CCR5, and CCR6 at high levels. Whereas MAIT cells were depleted from blood in patients with decompensated cirrhosis, MAIT cells were enriched in the peritoneal cavity at SBP diagnosis. Consistent with their chemokine receptor repertoire, activated MAIT cells preferentially migrated towards infected AF as compared to conventional T cells and were activated in a MR1-restricted fashion. Whereas circulating MAIT cells displayed features of immune exhaustion, peritoneal MAIT cells remained competent producers of interferon gamma and tumor necrosis factor in response to *E. coli.* In clinical association studies, the state of peritoneal MAIT activation correlated with the severity of systemic inflammation.

Conclusions Peritoneal MAIT are functionally competent inflammatory innate immune cells in decompensated cirrhosis, which accumulate during early SBP.

5.17 The alarmin IL-33 drives a ST2$^+$ Treg-mediated anti-inflammatory immune response during immune-mediated hepatitis

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Introduction Elevated levels of the alarmin IL-33 were shown in patients with chronic liver disease indicating an immunomodulatory role of IL-33 in hepatic inflammation. In Concanavalin (Con)A-induced immune-mediated hepatitis, IL-33 was released by necrotic hepatocytes and induced immune responses by signaling through the IL-33 receptor ST2. We have shown previously that IL-33 pre-treatment protected from immune-mediated hepatitis suggesting an immunosuppressive role of the IL-33/ST2 axis in liver disease.

Objectives Since regulatory T cells (Tregs) expressing ST2 respond to IL-33, we aimed at investigating the IL-33-driven ST2$^+$ Treg response in the inflamed liver.

Materials & methods To induce immune-mediated hepatitis, mice received ConA and were analyzed 24 hours later. To address the immunosuppressive effect of IL-33 on disease pathology, mice were treated with IL-33 on three days before ConA challenge. Foxp3$^+$ Tregs from IL-33-treated FIR-tiger mice

were adoptively transferred into C57BL/6 mice, which received ConA one day later.

Results In homeostasis, the frequency of $ST2^+$ $Foxp3^+$ Tregs was elevated in the liver compared to the spleen. Hepatic $ST2^+$ Tregs expressed higher levels of Foxp3 than $ST2^-$ Tregs and the frequencies of $ST2^+$ Tregs expressing ICOS, KLRG1, Ki-67, CTLA-4, TIGIT, CD39, CD73 and IL-10 were increased indicating an activated, proliferative and anti-inflammatory phenotype of this Treg subset in steady-state. In immune-mediated hepatitis, the frequency of $ST2^+$ Tregs was enhanced, which up-regulated expression of inhibitory molecules. Moreover, ST2-deficient mice developed more severe immune-mediated hepatitis despite an elevated frequency of $Foxp3^+$ Tregs in the inflamed liver, underlining the importance of the IL-33/ST2 axis for hepatic immune regulation. IL-33 pre-treatment before induction of hepatitis increased the frequency of activated hepatic $ST2^+$ Tregs compared to ConA-treated mice, which was associated with protection from liver disease. Moreover, transfer of IL-33-activated Tregs before ConA challenge potently suppressed immune-mediated hepatitis.

Conclusion The immune regulatory role of IL-33 in liver inflammation might be driven by activation, expansion and recruitment of a highly immunosuppressive Treg subset expressing ST2.

5.18 Autoimmune hepatitis in mice is enabled by insufficient deletion of autoreactive CD4 T cells and plasticity of Tregs

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Background The pathogenesis of autoimmune hepatitis (AIH) and the mechanisms responsible for the loss of tolerance to hepatic antigens are not clear.

Methods We generated a mouse model of AIH that is characterized by conditional hepatocellular expression of an MHC class II-restricted CD4 T cell epitope of lymphocytic choriomeningitis virus (GP61 – 80), and by abundance of cognate CD4 T cells recognizing GP61 – 80.

Results The mouse model is characterized by spontaneous development of CD4 T cell-driven hepatitis with typical hallmarks of human AIH, including elevated serum ALT, AST and IgG levels, lymphocytic periportal infiltrates with interface hepatitis, and antinuclear autoantibodies. GP61 – 80-specific T cells were abundantly present in the periphery, notably in the liver, due to the lack of thymic negative selection. As reported for human AIH (Bovensiepen and Schakat et al; J Immunol; in press), autoreactive CD4 effector T cells were characterized by IFNg and TNF co-production. Activation and pathogenic maturation of GP61 – 80-specific T cells seemed to occur locally in the liver within transiently formed portal ectopic lymphoid structures. Moreover, we observed a selective increase of plasticity and instability in GP61 – 80-specific regulatory T cells (Tregs) in the liver, but not of non-specific Tregs. Selective Treg instability was marked by IL-17 production, reduced Foxp3 expression and reduced demethylation of the *Foxp3* gene locus.

Conclusions Our findings indicate that AIH is driven locally in the liver by a selective failure of autoreactive Tregs to control the pathogenic maturation of autoreactive CD4 effector T cells producing IFNg and TNF.

5.19 Hippo signaling counter regulates early innate immunity in hepatocytes exposed to Hepatitis B virus

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Background & Aims Chronic hepatitis B virus (HBV) infection is a key risk factor for the development of hepatocellular carcinoma. Recent studies indicate potential relationships among HBV infection, Hippo signaling and innate immunity. Here, we investigated the interaction between Hippo signaling and HBV-triggered innate immune responses.

Methods Eligible pathways associated with HBV infection were analyzed using GSE69590 and GSE83148 data. Primary murine and human hepatocytes (PMHs and PHHs, respectively) were isolated and exposed to cell culture-derived HBV. Quantitative PCR, Western blotting and immunocytochemistry illustrated the activation and intracellular localization of YAP and NF-κB. Dual-luciferase reporter assay, chromatin immunoprecipitation, electrophoretic mobility shift assay and loss/gain of function experiments addressed the underlying mechanisms of this activation.

Results Reanalysis of gene arrays (GSE69590) identified HBV-related changes in Hippo and NF-κB signaling in HBV-infected PHH. Immunocytochemical staining and Western blot analysis showed a time-dependent nuclear translocation of YAP and NF-κB after HBV exposure in PMH. The application of TLR2 and MST1/2 inhibitors confirmed the involvement of the TLR2 and Hippo pathways in this model. PMHs isolated from *Irak4*-/- and *Myd88*-/- mice confirmed the relationship between TLR2 and Hippo signaling. Loss/gain of function experiments implied that YAP regulates IκBα expression. Functional investigations confirmed the regulation of *Nfkbia* promoter activity by the YAP/TEAD4 complex. IκBα fluctuation is a rapid innate immune control mechanism. Furthermore, administration of Pam3CSK4 or LPS to mice and the exposure of PHHs to HBV confirmed the relevance of the TLR2-MyD88-IRAK4-Hippo axis in innate immunity. Interestingly, gene array data (GSE83148) of HBV-infected patients and uninfected controls indicated that this TLR2-MyD88-IRAK4-Hippo axis is also relevant during chronic infection.

Conclusions In summary, we demonstrated that HBV is recognized by TLR2 which leads to increased NF-κB signaling and Hippo pathway activity to initiate and regulate rapid innate immune responses, respectively. The present data clearly indicate the importance of the TLR2-MyD88-IRAK4-Hippo axis in hepatic innate immune responses against HBV infection.

5.20 Cirrhosis and acute-on-chronic liver failure are linked with aberrant expression of co-stimulatory markers on T-cells

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Introduction/Question A hallmark of liver cirrhosis is the occurrence of systemic inflammation and immunosuppression in parallel. These two opposite conditions are strongly enhanced during development of acute-on-chronic liver failure (ACLF). This is demonstrated by phenotypic changes of innate immune cells as well as their elevated cytokine production. However, little is known about phenotypic changes in the adaptive immune compartment. Therefore, we aimed to characterize T cells of patients with liver cirrhosis and ACLF with regards to co-stimulatory and inhibitory marker expression and cytokine production.

Methods Peripheral blood mononuclear cells (PBMCs) were isolated from liver cirrhosis and ACLF patients and healthy donor buffy coats. For marker analysis, cells were stained for a total of 23 co-stimulatory and inhibitory cell surface markers. For cytokine response, PBMCs were stimulated with PMA/Ionomycin and stained intracellular for IFNgamma and TNFalpha. All samples were analyzed by flow cytometry.

Results Flow cytometric analysis of CD8+, CD4+ and regulatory (CD4+ CD25dim CD127-) T cells revealed changes in marker expression specific for the subtypes. Liver cirrhosis and ACLF patients showed lower frequencies of CD27+ CD8+ T cells, but higher frequencies of CD8+ T cells containing the inhibitory marker KLRG1. Frequencies of OX-40+ CD4+ T cells were elevated in patients but also higher frequencies of CD4+ T cells carrying the inhibitory marker 2B4 were observed. Regulatory T cells of patients were characterized as containing higher frequencies of CD28+, GITR+ and CTLA-4+ cells. All mentioned cell types displayed higher frequencies of the death receptor CD95 and the inhibitory receptor PDPN suggesting an overall situation of immunosuppression. In agreement, CD8+ and CD4+ T cells showed decreased or even lost capabilities of elevated IFNgamma and TNFalpha production after stimulation *in vitro*.

Conclusions Our results display dysfunctional T cell cytokine responses in liver cirrhosis and ACLF as a possible background for patients' susceptibility to infections. As a potential mechanism, we could identify an aberrant expression of co-stimulatory and inhibitory markers on all T cell subsets suggesting a shift towards immunosuppression and higher cell death rates. Combined immunophenotyping of all PBMCs could identify the distinct pathways responsible for immunosuppression in patients as potential targets for precise immunotherapy.

5.21 Persistence of a transcriptional chronic scar on HCV-specific CD8+ T cells after DAA-mediated antigen removal

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T-cell exhaustion, a functional impairment of virus-specific CD8+ T cells, is a hallmark of chronic HCV infection. Previously, we have reported that exhausted HCV-specific CD8+ T cells are comprised of terminally exhausted CD127-PD1hi and memory-like CD127+PD1+ subsets. Of note, memory-like HCV-specific CD8+ T cells are present during HCV infection and after DAA-mediated cure. Until now it is unclear to what extend memory-like HCV-specific CD8+ T cells resemble conventional memory or exhausted T cells. Additionally, the impact of DAA-mediated viral clearance and the accompanied loss of viral antigen recognition on the phenotype of memory-like HCV-specific CD8+ T cells is currently also unknown.

In order to define the fate of memory-like HCV-specific CD8+ T cells in chronic versus cured HCV infection, we conducted low-input RNAseq analyses of CD127/PD1-based HCV-specific CD8+ T-cell subsets obtained during and after chronic HCV infection targeting conserved and escaped epitopes (n = 5) and after spontaneous resolution of acute HCV infection (n = 3). Furthermore, we performed single cell RNAseq analyses of HCV-specific T cells from six chronically HCV-infected patients including longitudinal analyses after successful DAA therapy (n = 2).

Importantly, although memory-like HCV-specific CD8+ T cells exhibit some transcriptional characteristics of memory T cells, an exhausted signature is dominant even after DAA-mediated viral clearance. This suggests an imprinted exhausted T-cell fate. Thus, memory-like HCV-specific CD8+ T cells are clearly distinct from conventional memory T cells. In accordance with this, spatiotemporal analyses propose a progenitor/progeny relationship of memory-like and terminally exhausted HCV-specific CD8+ T cell subsets during chronic HCV infection. Noteworthy, HCV-specific CD8+ T cells targeting escaped epitopes also displayed an exhausted profile despite the lack of terminally exhausted subsets. Interestingly, however, the transcriptional pattern of these cells was unique compared to memory-like HCV-specific CD8+ T cells targeting conserved epitopes suggesting that duration of antigen recognition may have an impact on the transcriptional CD8+ T cell regulation.

In sum, our results show that chronic HCV infection is strictly linked to an "exhausted" T-cell differentiation that is not reverted by removal of viral antigen or loss of antigen recognition. This has potential implications for the control of re-infection and therapeutic vaccines.

5.22 Soluble CEACAM1 induces activation of STAT5, Foxp3 expression and proliferation of Tregs in DC- T cell cocultures

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Introduction CEACAM1 (carcinoembryonic antigen-related cell adhesion molecule 1) is a homophilic and heterophilic adhesion molecule that acts as an immune co-receptor on leukocytes. Soluble CEACAM1 (sCC1) was originally discovered as a serum marker in human patients with cholestasis and autoimmune liver disease. In murine immune-mediated hepatitis (Concanavalin A-induced hepatitis), CEACAM1 promotes IL-2-dependent Treg induction and stability, and *Ceacam1*$^{-/-}$ mice exhibit hyperinflammation and persistence of liver injury. This immune-regulatory function requires tight regulation of CEACAM1 isoform expression; on CD4$^+$ T cells, CEACAM1-S, the activatory isoform with a short cytoplasmic domain, fosters cytokine production and Treg induction. Contrary, its inhibitory isoform with two ITIMs and a long cytoplasmic tail (CEACAM1-L) interacts with co-inhibitory immune receptors (e.g. TIM-3) and limits activation.

Objectives The role of CEACAM1-ligation in hepatic immune regulation is unknown. The role of soluble CEACAM1/sCC1 in co-cultures of CD4$^+$ T cells and antigen-presenting cells (dendritic cells, DCs) for T cell activation and Treg induction is investigated.

Materials & methods In sera from human patients and mice, soluble CEACAM1 was detected by Western Blot. Cocultures from bone-marrow derived, FACS-sorted DCs and MACS-sorted T cells from CEACAM1-deficient and WT mice were analyzed by FACS, and cytokines were quantified by bead-based immunoassays and ELISAs.

Results CEACAM1 is detectable in sera of patients with advanced primary sclerosing cholangitis (PSC), and in sera of WT mice. In cocultures of CD4$^+$ T cells and dendritic cells, addition of sCC1 inhibits production of IL-2 by CD4$^+$ T cells and IL-12 by DCs, regardless of their CEACAM1 expression status. sCC1 strongly binds to DC-activated CD4$^+$CD25$^+$ T cells which results in phosphorylation of STAT5 and upregulation of Foxp3.

Conclusion sCC1 binds to activated T cells and induces STAT5 signaling and Foxp3$^+$ Tregs. On DCs, sCC1 binds to a heterophilic ligand, which leads to

reduction of IL-12 production. This immunomodulatory function of (s) CEACAM1 will be further explored *in vivo*.

5.23 Pyruvate kinase M2-deficiency in T cells leads to exacerbation of ConA hepatitis and alterations of T cell polarization

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Introduction In autoimmune liver disease, impaired homeostasis results from a dysbalance between regulatory T cells (Tregs) and T effector cells (Teff). Activated Teff cells undergo metabolic re-programming to preferential aerobic glycolysis known as Warburg Effect. Aerobic glycolysis is required for T cell growth and effector function. Contrary, Tregs rely on fatty acid oxidation. The Warburg effect and glycolytic switch are promoted by dimerization of pyruvate kinase M2 that acts as a cofactor for HIF-1a to induce transcription of inflammatory cytokines and glycolytic enzymes. These effects can be counterbalanced by allosteric tetramerization the PKM2. The cytosolic PKM2 tetramer has high enzymatic activity and ensures an intact glycolytic flux.

Objectives Deletion or inactivation of *Pkm2* in T cells should alter T cell polarization and T cell-mediated inflammation. Moreover, small molecule-induced tetramerization of PKM2 could restore immune homeostasis.

Materials & methods CD4cre*Pkm2fl/fl* mice ($PKM2^{\Delta CD4}$ mice) and controls were subjected to Concanavalin A (ConA)-induced hepatitis. ALT elevation and histology/immune histochemistry were employed to gauge disease outcome. Metabolism was analyzed with a Seahorse analyzer. In cocultures (hepatocytes (HC) + $CD4^+$ T cells), Treg induction was analyzed by FACS. Th1 and Th17 polarization of T cells was analyzed using FACS with and without the addition of PKM2 activators (TEPP46, stabilizes the PKM2 tetramer) and inhibitors (shikonin).

Results $PKM2^{\Delta CD4}$ mice exhibit exacerbation of ConA hepatitis. $PKM2^{\Delta CD4}$ Th1 and Th17 cells showed alteration in glycolytic activity. In HC-T cell cocultures and livers, enhanced Treg formation was observed in T cells/livers from $PKM2^{\Delta CD4}$ mice, whereas TEPP46 enhanced formation of $CD73^+$ Tregs *in vitro*. $PKM2^{\Delta CD4}$ Teff exhibited reduced CD73 expression. In Th17 cells, RORγt expression was reduced, whereas TEPP treatment restored RORγt expression *in vitro*.

Conclusion PKM2 is required for immune homeostasis by controlling the glycolytic switch in immune cell activation. PKM2 expression and activity affects Treg induction and Th17 cell polarization. The role of PKM2 activity and the different isomers in Treg induction and their suppressive properties and polarization of inflammatory Th17 cells is currently further investigated.

5.24 Metabolic programming of exhausted $CD8^+$ T cells in chronic viral hepatitis

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Exhausted T cells (T_{EX}) with limited function accumulate in chronic infections such as hepatitis B and -C virus infection. The prolonged exposure to viral antigen, the induction of co-inhibitory signals and the inflammatory milieu are thought to contribute to drive T_{EX}. T_{EX} are characterized by an increased inhibitory receptor expression and substantial alterations in their transcription profile. Regulation of energy metabolism has been suggested as a mechanism driving the dysfunction of exhausted T cells, however, the metabolic programming of HBV- and HCV-specific T cells in chronic infection and its links to T cell function remain unclear.

To address these important questions, we set out to profile key metabolic pathways involved in energy metabolism in patients with cHBV and cHCV using metabolism-directed flow cytometry and transcriptome profiling.

We found that in chronic infection, HCV-specific T cells display enhanced glucose uptake, but diminished mitochondrial polarization suggesting in comparison to HBV-specific T cells, a more severe type of exhaustion. Partial improvement of this phenotype was observed in patients with DAA therapy. Analysis of metabolic genes revealed an upregulation of *ACSS1/ACSS2* mRNA encoding for acetyl-CoA synthetase in HCV-specific T cells, suggesting higher ability to metabolize acetate as a potential anaplerotic TCA substrate. In agreement with this notion, addition of acetate to exhausted $CD8^+$ T cells from cHCV patients counteracted functional T cell exhaustion.

In sum, these results indicate that exhausted HBV- and HCV-specific $CD8^+$ T cells exhibit differential metabolic programming. Detailed understanding of metabolic regulation may allow metabolism-directed interventions to improve T cell function.

5.25 CD206 expression characterizes an inflammatory, resident human peritoneal macrophage subset in decompensated cirrhosis

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Question Peritoneal macrophages (PM) regulate inflammation and control bacterial infections in decompensated cirrhosis. The aim of this study was to characterize human PM heterogeneity and link PM activation with the outcome of spontaneous bacterial peritonitis (SBP).

Methods We phenotypically and functionally characterized human PM by flow cytometry, transcriptome-wide analysis of differential gene expression, *ex vivo* stimulation, and quantifying the soluble form of the mannose receptor CD206 in a cohort of patients with decompensated cirrhosis in absence and presence of SBP.

Results Employing CD206 surface expression, we identified subsets of human large (LPM) and small PM (SPM), which differed in granularity and maturation. PM subsets from patients with decompensated cirrhosis revealed discrete transcriptome clusters, comprising more than 4,000 differentially regulated genes involved in cell cycle, metabolism, self-renewal, and immune signaling. In contrast to SPM, LPM displayed a resident inflammatory phenotype, released higher levels of TNF after stimulation, and were less susceptible to tolerance induction. CD206 expression and release from LPM could be manipulated by incubating PM with TLR2/TLR6 and TLR4 agonists *in vitro*. Serial clinical samples revealed a relative depletion of LPM in the early phase of SBP followed by a recovery after treatment. Higher AF concentrations of sCD206 identified patients with SBP and indicated poor survival after 90 days.

Conclusions CD206 expression indicates a subset of mature, resident, inflammatory human PM. AF concentrations of its soluble form predict outcome of SBP.

5.26 In-vitro Activation and anti-viral Function of Innate NK cells in HEV Infection

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Background Hepatitis E virus (HEV) infection is a leading cause of acute hepatitis worldwide. While progressing asymptomatically and self-limiting in most patients, HEV infection can lead to severe courses especially in immunocompromised patients such as organ transplant recipients.Although humoral immunity against HEV have been extensively investigated, very few studies have evaluated the role of host cellular immune responses during acute and chronic HEV infection. Understanding the role that cellular immunity plays in clearing primary HEV infection may help to explain the limited durability of immune responses against HEV. Here we asked whether innate immune responses from natural killer (NK) cells contribute to antiviral immunity.

Methods Human hepatoma cell lines PLC/PRF/5 and Huh-7 were inoculated with a full-length HEV virus (MOI 0.5) and cultured for 7 days. HEV-infected cells were then co-cultured with PBMC"s from healthy donors for 24 h at an E:T ratio of 1:1. Viral replication was measured by real-time PCR and NK cells activation and function were analyzed by flow cytometry.

Results Co-culturing PBMCs with HEV-infected cells significantly decreased viral replication (PLC/PRF/5: 1.02e+07 vs. 8.99e+06; P = 0.0256; HuH-7: 1.74e+08 vs. 7,85e+07; P < 0.0001) and depleting NK cells from PBMCs diminished this anti-viral effect.

As assessed by flow cytometry, NK cells produced significantly more anti-viral cytokines such as interferon (IFN)-γ (PLC/PRF/5: 10.04% vs. 8.54%; P = 0.0304; HuH-7: 16.29% vs. 10.84%; P < 0.0001) and tumor necrosis factor (TNF)-α (PLC/PRF/5: 27.69% vs. 23.67%; P = 0.0120; HuH-7: 5.26% vs. 3.88%; P < 0.0001) in response to HEV infection.

However, co-culturing MAC-isolated NK cells with HEV-infected hepatoma cells showed neither a decrease in viral replication nor an activation and IFNγ-response of NK cells. Further isolation and depletion experiments revealed that NK cells need help by CD 14+monocytes in order to execute their anti-viral function against HEV.

Conclusions NK cells in the context of PBMC"s decrease HEV replication through IFNγ-secretion. However, NK cells need the presence of CD 14 monocytes in order to execute their anti-viral function.

5.27 Vergleich der Hepatitis E Seroprävalenz und Virämie zwischen HIV- und Transplant-Patienten

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Hintergrund Bei bis zu 50% der Immunsupprimierten kann eine Hepatitis E Virus (HEV) Infektion zu einer chronischen Hepatitis E bis hin zur Leberzirrhose und lebensbedrohlichen Komplikationen führen. Expositionsrisiko sowie Rate der Chronifizierung innerhalb verschiedener Gruppen Immunsupprimierter (wie HIV- oder Transplant-Patienten) sind bislang unzureichend untersucht. Ziel der vorliegenden Studie ist es die anti-HEV-Seroprävalenz und Virämie bei Transplantierten und Patienten mit einer HIV Infektion zu vergleichen.

Methoden Im Rahmen dieser Meta-Analyse wurde eine Literaturrecherche (Pubmed) durchgeführt und Studien von 1996 – 2019 in Bezug auf anti-HEV-Seroprävalenz und Virämie ausgewertet. Die Daten wurden nach Nation, Assay, Patientenkohorte und methodischer Qualität (nach JCBI) stratifiziert und mittels eines linearen Meta-Regressionsmodells analysiert.

Ergebnisse Es wurden 119 anti-HEV-IgG-Seroprävalenz Studien sowie 78 Studien mit Virämierate in die endgültige Analyse einbezogen. Bei Transplantatempfängern (14.629 Personen) lag die anti-HEV-IgG-Seroprävalenz bei 8% – 26% wohingegen bei HIV-positiven Patienten (23.692 Personen) 7% – 23% serologisch positiv waren. Die Virämierate, gemessen anhand positiver HEV-RNA Nachweise, war bei Transplantierten signifikant höher als bei HIV Patienten (1.16% vs. 0.4%; p < 0.05). Eine Subgruppenanalyse von Patienten mit erhöhten Leberwerten zeigte bei Transplantierten eine signifikant höhere Rate HEV-RNA Positiver als bei HIV Patienten (2.1% vs. 0.4%; p < 0.05).

Zusammenfassung Die Rate an positiven HEV-RNA Nachweisen – als Hinweis für einen chronischen Verlauf – war bei Transplantierten signifikant höher als bei HIV-positiven Patienten. Die anti-HEV Seroprävalenz hingegen – als Surrogatparameter für eine stattgehabte HEV Exposition – zeigte keinen Unterschied zwischen den beiden Gruppen. Eine Limitation der vorliegenden Untersuchung ist, dass keine detaillierten klinischen Daten, sowie Angaben hinsichtlich des Immunglobulin-Gesamtspiegels vorliegen. Diese Ergebnisse stützen die Annahme, dass Transplantierte im Gegensatz zu HIV-Patienten bei gleicher HEV Exposition einem höheren Risiko einer Chronifizierung ausgesetzt sind.

5.28 Soluble inflammatory molecules do not completely normalize despite early treatment of acute symptomatic hepatitis C

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Background and Aim Acute hepatitis C virus (HCV) infection has been associated with altered phenotypes and function of several immune cell populations and soluble inflammatory mediators (SIM). Successful treatment of chronic hepatitis C with DAA"s has been associated with alterations of SIM. However, no complete SIM restoration was observed. We hypothesized that early DAA treatment of symptomatic acute hepatitis C with DAAs may normalize most SIM – in contrast to treatment of persistent infection.

Methods In this study we made use of a unique cohort of 20 acute HCV patients who cleared HCV with a 6 weeks course of ledipasvir/sofosbuvir. As controls healthy donors (n = 20) and chronic non-cirrhotic patients (n = 25) were included. We applied a Proximity Extension assay (PEA) that uses high throughput, multiplex immunoassay measuring 92 proteins across 96 samples simultaneously.

Results Profound SIM alterations were observed in acute HCV patients with marked upregulation of IL-6, µPA, & TRAIL while few parameters were downregulated compared to healthy controls (e.g.s2B4). During the course of treatment and follow-up, the majority of SIM decreased with initiation of DAA but did not normalize until follow-up week 24 (e.g. CDCP1, IL-18). Of note, SIM that were downregulated before treatment remained suppressed while other parameters only declined to lower values during treatment and follow-up (e.g.Il-17).

Conclusions Antiviral treatment of acute hepatitis C leads to deep changes in the soluble inflammatory milieu. However, early treatment of very recent infection does not completely normalize altered SIM patterns. These findings may have implications for HCV re-exposure.

5.29 The Hepatitis C Virus influences CXC chemokine expression of infected host cells in response to IL-1β

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Background & aims The Hepatitis C Virus (HCV) employs several strategies to circumvent the antiviral immune response of its host. This also involves modulation of the local and systemic inflammatory response of the host. As it is known that serum levels of inflammatory cytokines such as Interleukin 1β (IL-1β) are upregulated in sera of HCV-infected patients, the present study analyses the impact of HCV on chemokine expression and in particular on the expression of CXCR2 ligands of its host cell in response to stimulation with IL-1β.

Methods CXCR2 ligands mRNA levels were determined in cells either harbouring the subgenomic replicon of HCV or in cells infected with HCV (HCVcc) by quantitative real time PCR (qRT-PCR). Cells were transfected with small interfering RNAs (siRNAs) and chemokine expression was determined. Activation of the NFκB subunit p65 was analysed by immunoblot. Binding of p65 to CXCL8 promoter region was determined by chromatin immunoprecipitation (ChIP).

Results CXCR2 ligand expression was upregulated in both replicon and HCVcc-infected cells. This HCV-mediated enhancement of chemokine expression was further strengthened upon stimulation with IL-1β. Phosphorylation of the NFκB subunit p65 and p65 binding to the respective binding site of the CXCL8 promoter were enhanced in subgenomic replicon cells in response to IL-1β stimulation. Consistently, knockdown of p65 expression resulted in decreased CXCR2 ligand expression in HCV-infected cells with and without IL-1β stimulation. Transforming growth factor β- activated kinase 1 (TAK1) knockdown led to decreased CXCR2 ligand expression at basal levels but did not affect the effects of HCV-infection and/or IL-1β stimulation. In addition, inhibitor studies suggest that regulation of CXCR2 ligand expression by HCV and/or IL-1β is independent from IKK, p38 MAPK and the proteinkinase B (Akt).

Conclusions The present study indicates that HCV enhances basal as well IL-1β-inducible expression of CXCR2 ligands and that this involves NFκB/p65-dependent regulation of gene expression, which is independent from TAK1, IKK, p38 MAPK and Akt.

5.30 Neuregulin-1 down-regulates expression of ErbB3 expression in hepatoma cell lines at the level of transcription

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Background and aims Previous studies from our group showed that HCV reduces the expression of ErbB3 in its host cell. As a result of this, the expression of EGFR, which is crucial for the viral entry, is enhanced, which suggests that HCV uses this rearrangement of the ErbB family members to improve its ability to enter the cell. Further investigations identified the ligand of ErbB3 receptor Neuregulin-1 to be responsible for its down-regulation and consequently triggering this circuit. The aim of the present study was to analyze whether down-regulation of ErbB3 by Nrg-1 also occurs in other human hepatoma cell lines and cells of other species like primary mouse hepatocytes and to assess whether this occurs at a transcriptional or post-transcriptional level.

Methods HepG2 cells were treated with human Nrg-1 and primary mouse hepatocytes with murine Nrg-1 at different concentrations and different times. The amount of ErbB3 mRNA in both cell types as well as the amount of pre-mRNA in HepG2 cells were measured by RT-PCR. Total protein lysates were analyzed by immunoblot with specific antibodies for ErbB3. Furthermore HepG2 cells were treated with Nrg-1 after adding specific inhibitors of the lysosomal and the proteasomal degradation system.

Results The Nrg-1 mediated down-regulation of ErbB3 is an effect which can be also observed in other human hepatoma cell lines, like HepG2. Like in Huh7 cells, the Nrg-1-stimulation caused a reduction of ErbB3 mRNA and protein expression of about 50% after 8 h. Also the analysis of pre-mRNA showed a decreased level with a minimum after 4 h of stimulation. In primary mouse hepatocytes there is a tendency of Nrg-1 decreasing ErbB3 on transcript level as well. On protein level we cannot show any differences between untreated and treated primary mouse hepatocytes. The inhibition of different pathways of the proteasomal and lysosomal degradation system in Nrg-1 stimulated HepG2 cells showed no alteration of these results so far.

Conclusion Evidence is provided that Nrg-1 reduces ErbB3 expression in human hepatoma cell lines, suggesting that this is not an affect, which only holds true for Huh7 cells, preferentially used as model system for HCVcc infection. Furthermore, the reduction of ErbB3 pre-mRNA suggests that the down-regulation occurs on the level of transcription. Concerning the degradation mechanism, we have found no evidence that it is dependent on clathrin-, dynamin- or rapamycin-mediated steps.

5.31 Real-world efficacy of EBR/GZR in HCV GT1 patients with multiple comorbidities and medications: results from the DHC-R

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Question Until now, the impact of comorbidities and DDIs on the outcome of elbasvir/grazoprevir (EBR/GZR) treatment is rather unclear. The present analysis of the DHC-R real-world cohort was aimed to assess the clinical relevance of co-morbidities and DDIs in patients (pts) with chronic HCV GT1 infection undergoing treatment with EBR/GZR.

Methods The DHC-R is an ongoing, prospective, observational cohort study. From September 2016 until July 2018, 992 pts with GT1 infection were treated at physician discretion with EBR/GZR +/- ribavirin (RBV) for 12 to 16 weeks in 130 medical practices and hospital outpatient departments. The present analysis was restricted to 613 pts who completed 12 or 24 weeks of follow-up or discontinued the treatment early; demographic data are shown for this ITT population. Sustained virologic response (SVR) data are shown for 599 pts (Per Protocol (PP) population). Comorbidities were documented and clinically relevant DDIs (co-administration of drugs contraindicated or may require dose adjustment/closer monitoring) were assessed based on information available at www.hep-druginteractions.org and the prescribing information for each drug.

Results This real-life cohort with 613 pts was predominantly male (59%), median age was 55 years and the majority (67%) was infected with HCV genotype 1b. 17% presented with liver cirrhosis. Pts were treated with EBR/GZR (93%) and with EBR/GZR+RBV (7%). 87% of pts reported 2641 comorbidities, while only 13% had no comorbidities. 1 – 3 comorbidities were observed in 33%, 4 – 6 comorbidities in 30%, >7 comorbidities in 24% of pts. The frequency of comorbidities was significantly higher in pts with GT1a vs. GT1b infection (94 vs. 84%, p = 0.005), in pts older than 50 – 70 years (90%) or > 70 years (92%) vs. < 50 years (82%) (p = 0.004 and 0.023) as well as in pts with cirrhosis vs. pts without cirrhosis (95 vs. 86%, p = 0.011). Comparable SVR rates were found when pts were analyzed according to the most frequent comorbidities. 65% of pts reported 1547 comedications, while 35% reported

no comedications at baseline. Overall, there was a low risk of DDIs with EBR/GZR (contraindicated 0.3% (2/613), DDIs requiring closer monitoring 7% (46/613)). In both groups, pts achieved an SVR of 100%.

Conclusions Despite a high frequency of comorbidities and comedications in pts with HCV GT1 infection clinically relevant DDIs are rare. Neither comorbidities nor DDIs seem to have an impact on SVR rates following EBR/GZR treatment.

5.32 The inflammatory mediators IL-1β and TNFα prevent CDCA-induced BSEP expression via induction of chemokines

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Question Cholestasis is a common complication in patients with sepsis and systemic inflammatory response syndrome. In this context IL1β and TNFα mediated downregulation of the expression of the bile salt export pump (BSEP) is known to be important (Luster et al., 1994, Roelofsen et al., 1995). However, the importance of the relevance of chemokines in this regard is unclear, and the elucidation of their significance for the inhibition of bile-salt-induced upregulation of the expression of BSEP is the subject of the present studies.

Methods primary hepatocytes isolated from murine liver and the human hepatoma cell line HepaRG were stimulated with different concentrations of TNFα and IL-1β, CDCA and SB 225002 either alone or in combination. Afterwards the gene expression of BSEP, CXCL 1 – 3 and 5 was determined by real-time PCR.

Results the results of the present study indicate that TNFα and IL-1β stimulate the expression of the CXCR2 ligands CXCL 1 – 3 and 5, which is even further enhanced in the presence of CDCA. Moreover in primary murine hepatocytes as well as in HepaRG, inhibition of the chemokine receptor CXCR2 by the compound SB225002 supposed to be specifically inhibit CXCR2 was able to block the inhibitory effects of IL-1β and partially of TNFa on CDCA-induced upregulation of BSEP expression.

Conclusion the data summarized herein suggest that TNFa and IL-1b both at least in part mediate their inhibitory effects on bile salt-induced upregulation of BSEP expression via induction of the expression of the CXCR2 ligands CXCL 1 – 3 and 5.

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5.33 Real-world efficacy of elbasvir/grazoprevir in HCV GT1 infected diabetics: results from the German Hepatitis C Registry

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Question Type 2 diabetes mellitus is a well-described extrahepatic manifestation of HCV infection. Here, we investigated the real-world effectiveness of EBR/GZR in patients (pts) without and with diabetes in a large GT1 cohort of the German Hepatitis C Registry (DHC-R). In addition, we evaluated the impact of HCV treatment on diabetes disease progression.

Methods From 09/2016 until 07/2018, 992 pts with GT1 infection were treated with EBR/GZR +/- RBV for 12 to 16 weeks in 130 medical practices and hospital outpatient departments in Germany. The present analysis was restricted to 879 and 93 pts without/with diabetes who completed follow-up. The primary outcome was per protocol sustained virologic response at 12 or 24 weeks post treatment (SVR12 or SVR24).

Results 879 pts (90%) without and 93 pts with diabetes (10%) showed the following characteristics: mean age 53 vs. 63 years, female gender 44 vs. 41%, BMI 26 vs. 29, GT1b 69 vs. 78% and cirrhosis 18 vs. 38%. In diabetics the rate of comorbidities was 2-fold higher than in pts without diabetes (8.3 vs. 3.7/patient). This was mainly related to a higher frequency of cardiovascular diseases (65 vs. 29%) and renal dysfunctions (24 vs. 7%) including hemodialysis (13 vs. 4%). In line with this observation, the use of outpatient medications was 2.5-fold higher in diabetic pts (5.6 vs. 2.1/patient) mainly by the intake of agents acting on renin/angiotensin (60 vs. 22%), beta blocking agents (39 vs. 19%), calcium channel blockers (25 vs. 10%), drugs for acid related disorders (41 vs. 15%) and diuretics (36 vs. 10%). PP SVR rates were similar in pts with (97.6%, 82/84) and without diabetes (97.5%, 766/786). Where available, HbA1c levels in pts with diabetes post SVR were compared to baseline and showed a lower percentage of 60% (3/5) vs. 100% (5/5) of pts with values exceeding the norm. HbA1c mean before treatment was 7.5% +/- 2.2% (N = 5) compared to 6.4% +/-1.1% (N = 5) at 24 weeks of follow-up. Likewise, the proportion of pts on antidiabetics decreased from 77% (65/84) at baseline to 56% (47/84) post SVR. Due to small patient numbers these results need to be interpreted with caution.

Conclusions In German real-world, treatment of GT1 infection with EBR/GZR results in a SVR rate of ≥ 97.5% in pts with/without diabetes. There seems to be a trend towards lower HbA1c levels and a reduction in intake of antidiabetics post SVR compared to baseline. This observation, however, needs to be confirmed in a larger study population.

5.34 Ribavirin-Dauertherapie als Therapieoption bei chronischer Hepatitis E Virus-Infektion

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Einleitung Chronische Verläufe von Hepatitis E Virus (HEV)-Infektionen bei Transplantatempfängern stellen ein zunehmendes klinisches Problem dar. Es ist davon auszugehen, dass 20 – 50% der HEV-Infektionen bei diesen Patienten chronisch verlaufen. Lebertransplantierte sind von einer fortschreitenden chronischen Hepatopathie und Transplantatversagen bedroht. 5 – 15% der Patienten erreichen unter einer off-label-Therapie mit Ribavirin kein dauerhaftes virologisches Ansprechen.

Fallbericht Bei einem 2008 aufgrund einer Leberzirrhose auf dem Boden einer chronischen Hepatitis B transplantierten Patienten wurde im Jahr 2012 im Alter von 73 Jahren eine HEV-Infektion (Genotyp 3) diagnostiziert. Unter Umstellung der Immunsuppression von Tacrolimus auf Mycophenolatmofetil konnte keine Ausheilung der chronischen Infektion erreicht werden. Eine off-label-Therapie mit Ribavirin (400 mg 1 – 0 – 1) wurde bei schlechter Verträglichkeit nach 2 Wochen abgebrochen. Unter einer an die Nierenfunktion angepassten Re-Therapie mit Ribavirin (200 mg 1 – 0 – 1) und Sofosbuvir

(400 mg 1 – 0 – 0) über 12 Wochen als individueller Heilversuch sowie Deeskalation der Immunsuppression kam es im Jahr 2016 zu einer Negativierung der HEV-RNA-PCR. In der Folge kam es 3 Monate nach Beendigung der Therapie zu einem Relapse. Im Jahr 2017 wurde bei signifikanter Leberwerterhöhung (GPT > 100 IU/ml) eine an die Nierenfunktion (KDIGO 4) angepasste Re-Therapie mit Ribavirin (200 mg 1 – 0 – 0; im Verlauf alle 2 Tage) initiiert, durch die ein deutlicher Abfall der Viruslast (Ausgangswert 3,9 Millionen Kopien/ml) mit im Verlauf negativer PCR und Normalisierung der Leberwerte erreicht werden konnte. Nach einer Therapiedauer von > 20 Monaten kam es unter Therapieauslass nach 5 Monaten wiederum zu einem Relapse. Seither wird Ribavirin dauerhaft (200 mg 1 – 0 – 0 alle 2 Tage) fortgeführt. Hierunter sind die Transaminasen bei guter Verträglichkeit ohne Auftreten einer Anämie und stabiler Nierenfunktion normalisiert.

Diskussion Eine Ribavirin-Dauertherapie in der niedrigsten effektiven Dosis könnte für ausgewählte Patienten mit chronischer HEV-Infektion ohne dauerhaftes virologisches Ansprechen eine Therapieoption darstellen.

5.35 Long-term course of a patient with overlap hepatitis C and IgG4-related cholangitis

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Background Hepatitis C virus (HCV) causes not only liver disease that may progress to cirrhosis but also can induce extrahepatic manifestations in nearly 75% involving other organs. IgG4-associated autoimmune cholangitis (AIC) has gained attention as a biliary manifestation of IgG4-related sclerosing disease, characterized by elevated serum IgG4-levels and infiltrates with IgG4-positive plasma cells.

Case report A 85-year old female patient with histologically-proven IgG4-associated cholagitis and HCV-induced compensated liver cirrhosis (Child A, genotype 1b) was sucessfully treated with sofosbuvir (SOF) and ledipasvir (LED) for 12 weeks. HCV-RNA declined from a baseline viral load of 455'800 IU/ml below detection 4 weeks after treatment. SVR 24 was achieved in the follow-up visits. Interestingly, IgG4-levels significantly decreased by antiviral treatment from 12"750 mg/l to 5"728 mg/l within four months. Next, the patient will be sheduled for immunosuppressive treatment of AIC with steroids and azathioprine. Patient"s characteristics, lab results, course of both diseases, and treatment were documented from November 2014 to October 2019.

Conclusions Hepatitis C virus can induce several autoimmune disorders; in our case overlap to IgG4-related cholangitis was diagnosed. This is the first reported case of IgG4-related cholagitis improving by antiviral treatment.

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