33. Jahrestagung der Deutschen Arbeitsgemeinschaft zum Studium der Leber

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1. Fibrogenesis

1.1 Der duale CCR2/CCR5 Antagonist Cenicriviroc reduziert Steatohepatitis und Fibrose durch Inhibition infiltrierender pro-inflammatorischer CCR2+ Monozyten in Mausmodellen der chronischen Leberschädigung

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Hintergrund: Inflammatorische Monozyten-abhängige Makrophagen (Mo-Mϕ) sind maßgeblich an Progression und Regression der Leberfibrose beteiligt. In der Maus wie im Menschen werden pro-inflammatorische Monozyten über den CCL2-CCR2 Chemokin-Signalweg in die Leber rekrutiert und stimulieren dort die hepatische Fibrosierung. Mittels des oral verfügbaren CCR2/CCR5 Antagonisten Cenicriviroc (CVC), der sich aktuell in der klinischen Phase 2b zur Therapie von NASH und Leberfibrose befindet, haben wir die Wirksamkeit der Inhibition infiltrierender pro-inflammatorischer Monozyten in akuten und chronischen Leberschadensmodellen der Maus untersucht. **Methoden:** Die Wirksamkeit von CVC wurde in insgesamt fünf verschiedenen Modellen in männlichen C57BL/6J Wildtyp-Mäusen analysiert: im akut toxischen Schaden durch Tetrachlorkohlenstoff (CCl4) und sowohl in Progression als auch Regression im chronisch toxischen Schaden durch CCl4 (6 Wochen) und in der Steatohepatitis durch Methionin-Cholin defiziente (MCD) Diät (8 Wochen). An primären murinen Zellen wurde zudem in vitro der Einfuss von CVC auf die Aktivierung und den Metabolismus von Knochenmarksmonozyten und Hepatozyten untersucht. **Ergebnisse:** Sowohl CCl4 Schädigung als auch MCD Diät führen zu einer massiven CCR2-vermittelten Anreicherung proinflammatorischer Ly-6C+ Mo-Mϕ in der Leber. Im akuten CCl4 Modell reduziert die orale Gabe von CVC Ly-6C+ Monozyten im Blut ($p < 0{,}01$) und in der Leber ($p < 0{,}001$). Ebenso konnte die therapeutische CVC Applikation in den letzten 3 Wochen von insgesamt 6 Wochen CCl4 Schädigung Blutmonozyten ($p < 0{,}05$) und hepatische Mϕ Level ($p < 0{,}05$) signifikant reduzieren. Die deutlichsten Ergebnisse zeigte das steatohepatitische Schadensmodell, in dem die therapeutische Gabe von CVC in den letzten 4 Wochen von insgesamt 8 Wochen MCD Diät die Ly-6C+ Mo-Mϕ in der Leber signifikant ($p < 0{,}001$) reduzierte. Die Gabe von CVC reduzierte signifikant sowohl Steatohepatitis als auch Fibrose im MCD Modell. Dahingegen werden Reparaturmechanismen in Regressionsphasen des CCl4 oder MCD Modells nicht durch CVC-Gabe gestört. Die antifibrotische Wirkung von CVC in Mausmodellen scheint hauptsächlich auf der Mo-Mϕ Inhibition zu beruhen, weil CVC selbst weder

die Polarisierung von Knochenmarksmonozyten noch den Fettmetabolismus von Hepatozyten in vitro beeinflusst. **Schlussfolgerungen:** In Mausmodellen des akuten und chronischen Leberschadens inhibiert der oral verfügbare CCR2/CCR5 Antagonist CVC effektiv die Infiltration pro-inflammatorischer Monozyten in die Leber. Vor allem die therapeutische Applikation im steatohepatitischen Schadensmodell verbessert sowohl Leberentzündung als auch -fibrose, so dass CVC eine vielversprechende therapeutische Option zur Behandlung von NASH Patienten darstellen könnte.

1.2 In vivo imaging of liver injury and regeneration by functional two-photon microscopy

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Intravital two-photon based imaging allows real-time observation of biological processes. Videos can be recorded at sub-cellular resolution up to 200 nm. Although the mechanisms of acetaminophen (APAP) induced liver injury are intensively studied, this technique gave us surprising insights both during the destruction and the regeneration processes. Videos recorded directly after administration of a toxic dose of APAP (300 mg/kg) identified two different scenarios of cell death. Hepatocytes which are close the central veins undergo necroptosis as already described in literatures. Surprisingly, the outer layer of the pericentral hepatocytes are killed by in dependent mechanism which is mediated by bile salt overload. First, dilatation of the bile canaliculi was observed within 30 min after APAP administration. Subsequently, the widened bile canaliculi form protrusions into the adjacent hepatocytes. These protrusions continue to increase in size and finally burst leading to bile regurgitation into hepatocytes and cell death. Interestingly, similar scenario was observed in cholestasis induced by bile duct ligation. The regeneration process after a single APAP challenge is characterized by a transient activation of hepatic stellate cells (HSCs) which reach to the peak on day two and disappear between days four and six after APAP injection. This was associated with macrophages infiltration into the dead cell area. In contrast to the situation during fibrosis resolution, the activated HSCs did not undergo apoptosis following regeneration from an acute challenge. Although work for a formal proof is still in progress, it seems as if the infiltrating macrophages interact with and finally phagocytose the activated HSCs in the dead cell area. This stimulated us to deplete macrophages by repeated administration of clodronate. Removal of macrophages lead to prolonged activation of HSCs. Interestingly, shortly after macrophages depletion backup infiltration of CD45+ immune cells was observed followed by apoptosis of HSCs. In conclusion, the direct observation of cellular and sub-cellular events in the living liver allows insights into the sequence of pathophysiological events which are difficult to obtain by conventional methods.

1.3 Interleukin-13 Depletion normalisiert den enterohepatischen Gallensäurekreislauf in Abcb4 knockout Mäusen

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Einleitung: Patienten mit IgG4-assoziierter Cholangitis weisen erhöhte biliäre Konzentrationen Th2-spezifischer Zytokine auf. In diesem Zusammenhang wurde gezeigt, dass insbesondere Interleukin-13 (IL-13) die Tight-Junction-assoziierte biliäre Epithelzell Barrierefunktion vermindert. Im Abcb4-knockout-Mausmodell für die intrahepatische Cholestase erfolgt die progressive hepatische Parenchymschädigung aufgrund der verminderten biliären Epithelzell Barrierefunktion. Mit dem vorliegenden Projekt soll der therapeutische Nutzen einer Depletion von IL-13 im Abcb4-knockout Maus Modell analysiert werden. **Methoden:** Durch Kreuzen von Abcb4-/- und IL-13-/- Mäusen wurden Doppel-Knockout-Hybride gezüchtet und weibliche Tiere in den Altersstufen 8, 26 und 52 Wochen untersucht (jeweils n = 10). Die Leberintegrität wurde mittels histologischer- und serologischer Tests analysiert. Auf molekularer und zellbiologischer Ebene wurden Serum sowie Gewebe aus Leber und Ileum mithilfe von Western Blot, Gallensäureanalyse, Hydroxyprolin-Analyse, Immunhistochemie und qRT-PCR untersucht. **Ergebnisse:** Mit der vorliegenden Studie wurde gezeigt, dass das Ausschalten von IL-13 im Abcb4-Knockout Maus Modell für Cholestase eine ca. 10-fache Verringerung der Serum Gallensäurekonzentration zur Folge hat. Die Gallengangsarchitektur und Zell-Zell-Kontakte sind in Abcb4-/-/IL-13-/- Doppel-Knockout-Mäusen deutlich weniger gestört als in Abcb4-/- Mäusen. Der in der Abcb4-/- Maus gestörte enterohepatische Gallensäurekreislauf und die Schädigung der Leber und des Ileums werden durch IL-13 Depletion normalisiert. Darüber hinaus wurde eine Verbesserung der Leberintegrität (ALT), verminderte Apoptose des Parenchyms bei jungen Mäusen und eine verminderte Fibrose im Langzeitverlauf beobachtet. **Diskussion:** Die durch den IL-13 Knockout verbesserte Barrierefunktion der Gallengänge bewirkt eine Normalisierung der pathologischen Gallensäurespiegel im Blut der Abcb4-Mäuse und verbessert die Leberpathologie. Darüber hinaus erweitert die vorliegende Studie das molekularbiologische Verständnis des durch IL-13 beeinflussten Gallensäuremetabolismus und der damit verbundenen hepatischen Cholestase und könnte in Verbindung mit anderen Therapiestrategien eine translationale Anwendung finden.

1.4 Eine Transgen induzierte unfolded protein response (UPR) steigert Leberschädigung und Karzinogenese im Cholestase-Maus Modell

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Einleitung: Chronisch infizierte Hepatitis B Patienten haben unter Immunsuppression ein erhöhtes Risiko, eine fibrosierende cholestatische Hepatitis zu entwickeln [1]. Ein Teil dieser Patienten zeigt eine signifikant erhöhte Expression der Hepatitis B Virus (HBV) Oberflächenproteine (HBs) [2]. In klinischen Studien und Laborversuchen wurden direkte zelltoxische Effekte bei HBs Expression gezeigt [3]. **Methoden:** In Hybriden von HBs-transgenen Mäusen und Abcb4-/–Mäusen wurden in Serum und Leber per qRT-PCR, Serum-Analytik, Western Blot und histologisch Aminotransferasen, Gallensäuren, Inflammation und Fibrogenese, UPR sowie Tumorgenese assoziierte Signalwege analysiert. **Ergebnisse:** Mit der vorliegenden Studie wurde die Verstärkung der cholestatischen Lebererkrankung und Tumorgenese durch transgene Expression von HBs in Abcb4-/- Mäusen gezeigt. Erhöhte Serum Gallensäure Konzentrationen sowie verminderte Level der protektiven Gallensäure TUDCA gehen mit erhöhter Serum-ALT Aktivität und verstärkter Fibrogenese einher. Auch die HBs induzierte UPR wird durch die zusätzliche Cholestase verstärkt, obwohl weniger HBs im Hybrid-Modell exprimiert wird. **Schlussfolgerung:** Interessanterweise verstärkt die transgene Expression von HBs auch ohne virale Infektion die intrahepatische Cholestase und Karzinogenese in Abcb4-knockout Mäusen. Dieser Befund legt nahe, dass eine Senkung der HBs Expression für chronisch HBV infizierte Patienten eine Verbesserung der Therapie und Prognose bedeuten könnte. **References:**
[1] Davies SE, Portmann BC, O'Grady JG, Aldis PM, Chaggar K, Alexander GJ, Williams R. Hepatology. 1991; 13: 150 – 157. [2] Lau JY, Bain VG, Davies SE, O'Grady JG, Alberti A, Alexander GJ, Williams R. Gastroenterology. 1992; 102: 956 – 962. [3] Churin Y, Roderfeld M, Stiefel J, Wurger T, Schroder D, Matono T, Mollenkopf HJ, Montalbano R, Pompaiah M, Reifenberg K, Zahner D, Ocker M, Gerlich W, Glebe D, Roeb E. PLoS One. 2014; 9: e90608.

1.5 Iso-Alpha Acids (IAA) from hops (Humulus lupulus) inhibit hepatic steatosis, inflammation and fibrosis

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Chronic alcohol consumption is one of the leading causes of liver disease world-wide. Epidemiological and animal studies indicate that in addition to the amount of alcohol also the type of consumed alcoholic beverages impacts liver injury with beer being less harmful for the liver than intake of spirits. Iso-alpha-acids (IAA), the main bitter acids present in hops, are constituted during wort boiling with hop, thereby providing beer with its typical bitter taste and foam stability. The aim of this study was to analyze whether IAA beneficially affect the pathogenesis of alcoholic or non-alcoholic (fatty) liver disease. **Methods:** IAA were applied to mice in combination with different models of liver injury: (i) high fat, non-alcoholic steatohepatitis (NASH) inducing diet (HFD) in mice, (ii) combined HFD and alcohol application to mice, and (iii) toxic liver injury induced with CCl4 in rats. Furthermore, IAA effects on primary human hepatocytes (PHH) and hepatic stellate cells (HSC) were analyzed in vitro. **Results:** IAA significantly reduced HFD or HFD/alcohol induced hepatic steatosis. Reduced expression of PPAR-gamma and key enzymes of lipid synthesis as well as increased expression of PPAR-alpha, indicative for increased lipid combustion, were identified as underlying mechanisms. Analysis of hepatic HMOX1 expression and MDA levels showed reduced oxidative stress in IAA treated mice, which was paralleled by reduced serum transaminase levels, reduced activation of the JNK-pathway and pro-inflammatory gene expression and immune cell infiltration, as well as reduced HSC activation and profibrogenic gene expression. Fitting to this, IAA dose-dependently inhibited cellular lipid accumulation, PPAR-gamma expression and oxidative stress in PHH in an in vitro model of hepatic steatosis. Furthermore, IAA inhibited HSC activation as well as proinflammatory and profibrogenic gene expression in already activated HSC in vitro. Also in the CCl4 model, IAA significantly inhibited cellular damage, oxidative stress, inflammation and fibrosis. **Conclusion:** IAA inhibit different pathophysiological steps of disease progression in various models of chronic liver disease. IAA content in beer may explain why it is less harmful for the liver compared to pure alcohol, but certainly, alcohol content in beer surpasses the beneficial IAA effect. However, together with previous studies, which demonstrated the safety of even long term application of IAA in men, our data suggest IAA as promising therapeutic agent for the prevention and treatment of (non)alcoholic (fatty) liver disease.

1.6 Increased expression of pregnancy-associated plasma protein-A (PAPP-A) in hepatic stellate cells correlates with hepatic fibrosis and can be detected in the serum of patients with liver disease

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Pregnancy-associated plasma protein-A (PAPP-A) was firstly discovered as a placental protein present in the circulation of pregnant women. PAPP-A is a metalloproteinase that specifically cleaves insulin-like growth factor (IGF) binding proteins (IGFBPs). It binds tightly to glycosaminoglycans present on the cell surface and thus functions as a growth-promoting enzyme, releasing bioactive IGF in close proximity to the IGF receptor. Recently, we have discovered PAPP-A as a tumor promotor in hepatocellular carcinoma applying causal modeling (PLoS Comput Biol. 2015;11(5):e1004293). Although the IGF-system is known to play a critical role in liver fibrosis, no further information existed regarding the expression and function of PAPP-A in chronic liver disease. Therefore, the aim of this study was to analyze the expression and function of PAPPA in liver fibrosis. **Methods and Results:** Hepatic PAPP-A expression was significantly increased in different murine models of hepatic fibrosis (toxic liver injury with TAA or CCl4, bile duct ligation and dietary models of non-alcoholic fatty liver disease). Furthermore, PAPPA expression revealed a significant correlation with collagen I and alpha-smooth-muscle expression in human hepatic tissue specimens from patients with chronic liver disease. Moreover, PAPPA serum levels were significantly increased in patients with cirrhosis as compared to individuals without liver disease. Fitting to this, PAPPA expression significantly increased in hepatic stellate cells (HSC) during in vitro activation, and suppression of PAPPA expression in activated HSC with siRNA resulted in reduced PAPPA secretion into the supernatant. Furthermore, PAPPA suppressed HSCs showed significantly reduced proliferation as compared to HSC transfected with control siRNA. In addition to PAPP-A, we newly discovered strong expression of IGFBP-4 in activated human HSCs, and analysis of human activated HSC isolated from 14 different donors revealed a significant correlation between PAPP-A and IGFBP-4 expression. **Summary and Conclusion:** PAPP-A expression and secretion increase during activation of HSC, and our data suggest that this leads to an autocrine induction of HSC proliferation, potentially via cleavage of IGFBP-4 and consecutive release of (bioactive) IGF. This indicates PAPP-A as novel target for anti-fibrogenic therapy. Moreover, activated HSC appear to be the major source of PAPP-A in diseased livers, and herewith, circulating PAPP-A levels appear as potential novel biomarker for hepatic fibrosis.

1.7 ABCB5+ mesenchymal stem cell transplantation in a chronic liver disease mouse model

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Aim: ABCB5+ cells are mesenchymal stem cells isolated from human skin. Stem cells in general have the capacity to differentiate in any type of cell. Their therapeutic potential has already been shown by significantly reducing chronic venous ulcers and minimizing inflammation due to macrophage inhibition. Due to organ-donor limitations, stem cells provide a promising therapy option for patients with chronic/end stage liver disease since there is no other chance of survival than liver transplantation. In previous studies, little side effects in short time observations and no graft versus host effects have been monitored. **Methods:** Mdr2-/- mice develop different stages of liver damage from fibrosis with inflammation to HCC. The mice were immunosuppressed by implanting an osmotic pump steadily releasing Tacrolimus. 24 hours post implantation, 5 × 105 ABCB5+ cells were injected into the tail vein. After two days, livers were resected to detect ABCB5+ cells in the liver, and after two and four weeks to measure any therapeutic effect in the damaged livers of Mdr2-/-mice. Changes or improvements of liver damage, in particular on inflammatory and fibrotic markers, were evaluated on mRNA level, tissue morphology and liver parameters in plasma. **Results:** Application of ABCB5+ stem cells show no toxic effect on liver plasma parameters (ALT, AST) and a tendency of reduced alkaline phosphatase. Preliminary results indicate no obvious difference between untreated Mdr2-/- mice and stem cell transplanted animals after 2 and 4 weeks when analysing mRNA levels of fibrosis and inflammation markers. However, after two weeks of ABCB5+ treatment, there is a tendency of fibrosis and inflammation reduction, as analyzed by Sirius red and macrophage marker S100A4 staining, and after 2 weeks, this reduction is 4% lower than in controls, but after 4 weeks a 20% decrease. **Conclusions:** The mice tolerated the ABCB5+ stem cells very well and we see a small beneficial effect, which will be further consolidated. An increasing cell number may help to approve the shown tendency and we do further analyses regarding fibrosis and progressed inflammation (e.g. hydroxyproline assay to quantify the collagen concentration).

1.8 Anti-fibrotic and anti-inflammatory consequences of TGF-β2 silencing in biliary liver disease

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Aim: We targeted TGF-β2 expression using antisense oligonucleotides (AONs) in MDR2-KO mice to attenuate fibrogenesis and translated our findings to human PBC and PSC patients. **Methods:** In 16 weeks old MDR2-KO mice, TGF-β2 expression was targeted using AONs for 4 weeks. Therapeutic efficacy was evaluated by cell-type specific immunofluorescent analysis of AON biodistribution, tissue morphology, and liver parameters. Expression of TGF-β2 and markers for fibrosis and inflammation were investigated by RT-PCR, immunoblot and immunohistochemistry (IHC). TgfB2 mRNA and TGF-β2 expression were determined in livers and serum of PSC and PBC patients using RT-PCR, IHC and ELISA and correlated to clinicopathological parameters. **Results:** In MDR2-KO tissue, TGF-β2 was expressed in fibroblasts and areas of proliferating bile ducts; whereby the AONs accumulated in non-parenchymal cells. While ALT, AST and body weight were not affected, TgfB2 levels, hydroxyproline content, collagen deposition and αSMA protein expression were downregulated in mouse livers upon AON treatment. In line with an induction of PparG, inflammatory infiltrates were significantly reduced. According to MDR2-KO mice, TGFβ2 expression was upregulated in PSC and PBC patients compared to normal livers. Especially PBC patients (GSE79850) classified with high risk (no treatment response, liver transplantation requirement) showed increased TGFB2 expression. Preliminary results indicate that in PBC and PSC, TGF-β2 is also localized in areas of proliferation bile ducts. TGF-β2 protein expression in corresponding sera is currently investigated. **Conclusions:** TGF-β2-directed AON application attenuated fibrogenesis and inflammation in MDR2-KO mice. Corresponding upregulation of TGF-β2 in PSC and PBC patients unveils TGF-β2 as an interesting target for treatment of human biliary diseases.

1.9 aP2-specific inactivation of fatty acid transport protein 4 in mice sensitizes hepatic fibrogenesis under methionine and choline-deficient diet

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Background/Aims: Fatty acid transport Protein 4 (FATP4) catalyzes activation of fatty acids into specific complex lipids, such as phospholipids and sphingolipids. It is known that FATP4 plays a major role in the skin by altering ceramide metabolism. We have previously shown that adipocyte-specific FATP4 deficiency (by using aP2-Cre FATP4KO mice) sensitizes with high-fat diet for an increase of body and adipose tissue weights concomitant with a manifestation of fatty liver. The latter could be due to an overspill of adipose fats or a presumed FATP4 effect on hepatic stellate cells and/or macrophages which are known to also express aP2. We therefore investigated the latter possibility by feeding aP2-Cre FATP4KO mice with methionine and choline-deficient (MCD) diet which is a fatty liver model with severe fibrosis and inflammation. **Methods:** WT and aP2-Cre FATP4KO female mice at ~12 months old were fed with MCD diet for 4 weeks. Functional assays included transaminases, qRT-PCR, Western blot, histology, and immunohistochemistry (IHC). **Results:** MCD diet feeding of WT and KO mice caused the same body weight loss, serum ALT and caspase 3 elevation, the extent of fatty liver, increased vimentin protein expression as well as elevated mRNA expression of PPARγ, TIMP1, smad7, and collagen 1a1/3a1/4a1. However, compared with MCD-fed WT MCD-fed KO mice exhibited aggravated hepatic fibrogenic response as revealed by an increase of Sirius-Red staining, α-smooth muscle actin by IHC and Western blot as well as mRNA expression of plasminogen activator inhibitor-1. Such effects were not observed for inflammatory markers including F4/80 by IHC and TNF-α mRNA expression, suggesting no involvement of macrophages in the observed MCD and FATP4 response. Notably, MCD feeding of WT mice caused a marked increase of mRNA and protein expression of a structural protein E-cadherin, and this was not observed in MCD-fed KO mice. **Conclusions:** FATP4 deficiency in an aP2-specific manner sensitized by MCD diet for increased hepatic fibrogenesis in part by the failure in upregulating cell-to-cell adhesion protein namely E-cadherin. Mechanism for this may involve FATP4 catalysis of specific phospholipids whereby lipidomics analyses of these liver samples are planned in our laboratory. Our results provide the first evidence for possible role of FATP4 in hepatic fibrogenesis, and further experiments using isolated hepatic stellate cells are necessary to verify this.

1.10 Biopsy-based analysis of the prosteatotic TM6SF2 p.E167K and PNPLA3 p.I148 M gene variants as potential modifiers of Wilson disease

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Objectives and study: Wilson disease (WD) is a rare liver disease caused by mutations of the ATP7B gene. The presence of genetic modifiers of WD has for long been suspected, however no common polymorphism affecting the progression of this disorder in pediatric patients has been detected so far. Previous studies demonstrated that the prosteatotic gene variants, PNPLA3 p.I148 M and TM6SF2 p.E167K are associated with increased liver injury in adults with chronic liver diseases. Here we investigate these variants as potential modifiers of liver injury in a large cohort of pediatric WD patients. **Methods:** Overall, we recruited 79 children (age 9 ± 5 years, 10 pairs of sibs) with WD among whom 5 required liver transplantation. Genotyping of the TM6SF2 rs58542926 and PNPLA3 rs738409 polymorphisms was performed using TaqMan assays with fluorescence detection. Liver biopsy was performed in 59 children (75%). Steatosis (micro- and macrovesicular) and fibrosis were quantified by pathologists blinded to the genotyping results. All unrelated patients and randomly selected one sib from each pair were included in analyses: association tests were calculated in contingency tables, continuous variables were compared using Mann-Whitney or ANOVA tests. **Results:** Among the biopsied patients, 57% displayed steatosis grade 1 and 20% presented with steatosis grade 2. Fibrosis stages 2 – 4 were present in 74% of patients, whereas only 5% showed no fibrosis at liver biopsy. The genotype frequencies of the TM6SF2 p.E167K and PNPLA3 p.I148 M polymorphisms did neither differ from Caucasian population frequencies nor deviate from HWE ($P > 0.05$). In total, 50% of patients carried at least one copy of the TM6SF2 or PNPLA3 risk allele. The presence of the minor TM6SF2 allele was associated with an increased risk of developing fibrosis stage ≥ 2 (OR = 9.36, 95% CI 0.51 – 170.92, $P = 0.04$): All carriers of the TM6SF2 risk allele displayed fibrosis stages ≥ 2 at biopsy. This variant was however not associated with either liver steatosis or with liver function tests (both $P > 0.05$). Although the PNPLA3 minor allele was associated with none of the histologic traits ($P > 0.05$), its carriers had significantly ($P = 0.01$) increased INR possibly reflecting enhanced liver injury. In total, 4 out 5 (80%) transplanted children carried at least one copy of the PNPLA3 or TM6SF2 risk alleles. **Conclusions:** Our results suggest that the TM6SF2 p.E167K, and to a lesser extent, PNPLA3 p.I148 M polymorphisms might modulate liver injury in children with WD. Since fibrosis seems to be frequent in pediatric WD, testing of the TM6SF2 variant could assist in detecting patients with an increased risk of disease progression.

1.11 Presence of the MBOAT7 rs641738 variant might enhance liver fibrosis in patients with fatty liver: analysis of the German NAFLD CSG cohort

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Background and aims: Familial clustering of fatty liver underscores the role of genetic predisposition in the development of this condition. Recently the MBOAT7 rs641738 polymorphism has been detected as a novel prosteatotic variant (Mancina et al. Gastroenterology 2016) together with the established PNPLA3 p.I148 M and TM6SF2 p.E167K variants. In the current study we investigated the association of this variant with markers of liver injury in NAFLD patients. **Patients and methods:** Overall, we recruited 515 patients with NAFLD (age 16 – 88 years, 280 females) in 8 German tertiary referral centers. Liver biopsies were performed in 320 patients. PCR-based assays were used to genotype the MBOAT7 rs641738 polymorphism. Serum concentrations of CK18-M30 fragment were measured by ELISA in 135 patients. **Results:** The MBOAT7 genotype distribution was: [CC] n = 159, [CT] n = 242, and [TT] n = 114. Among 320 biopsied individuals 57% had steatosis grades 2 or 3. Fibrosis stage F2 or higher was present in 30% of patients, while liver cirrhosis was detected in 21 (6%) individuals. There was no difference in MBOAT7 genotype distribution in NAFLD patients with or without liver biopsy. On the other hand, the presence of this variant was associated with an increased risk of fibrosis (common OR = 1.44, P = 0.04), whilst not affecting serum ALT or ASL activities or CK18-M30 levels or liver steatosis (P > 0.05). In contrast to studies in patients with alcoholic liver disease (Buch S et al., Nat Genet 2015), we did not detect an association of this variant with liver cirrhosis in our cohort. **Conclusions:** The MBOAT7 genotype might be associated with early stages of liver fibrosis in patients with NAFLD. Given the low number of individuals with advanced fibrosis in our cohort, genetic analyses of larger groups of patients with more prominent liver disease are necessary to delineate the association of the MBOAT7 variant with hepatic injury in the setting of NAFLD.

1.12 Die microRNA „miR-122“ spielt keine Rolle bei der Aktivierung von hepatischen Sternzellen

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Einleitung: miR-122, die rund 70% der gesamten miRNAs in der Leber ausmacht, wird spezifisch in Hepatozyten exprimiert und ist mit verschiedenen akuten und chronischen Lebererkrankungen assoziiert. Durch mehrere Arbeiten wurde eine Minderexpression der miR-122 in hepatischen Sternzellen (HSC) gezeigt, die mittels Iohexol-Gradientenzentrifugation isoliert und in vitro aktiviert wurden (u. a. Li et al. J Hepatology 2013). Jedoch ist in derartigen HSC-Isolaten eine deutliche Hepatozyten-Kontamination feststellbar. (Maderacke et al. Nat Protoc 2015). Wir haben daher analysiert, ob die beschriebene Regulation der miR-122 während der HSC-Aktivierung durch eine initiale Kontamination mit Hepatozyten hervorgerufen wird, welche im Verlauf der spezifischen HSC-Kultivierungsbedingung verloren geht. **Methodik:** Es wurde die Reinheit sowie die miR-122-Expression von HSC vergleichend untersucht, die mittels Iohexol-Gradientenzentrifugation alleine (HSCG) oder mit zusätzlicher FACS-Aufreinigung (HSCG+S) aus Mauslebern isoliert wurden. Die HSC-Isolate wurden zusätzlich über 5 Tage in vitro aktiviert, jeweils die miR-122-Expression vor und nach Aktivierung gemessen und diese mit der Expression von Hepatozyten-Markern (Albumin, HNF4α) korreliert. **Resultate:** HSCG zeigten direkt nach der Isolation gegenüber HSCG+S eine höhere Expression von Albumin (23-fach), HNF4α (35-fach) und von miR-122 (21-fach). Nach fünftägiger Aktivierung war sowohl die miR-122-Expression als auch die Expression der Hepatozyten-Marker in transaktivierten HSCG deutlich verringert und lag auf einem vergleichbaren Niveau wie HSCG+S an Tag 0 bzw. Tag 5. Unsere Daten legen daher eine initiale Hepatozyten-Kontamination in HSCG nahe, welche während der Aktivierung in-vitro verloren geht. In aufgereinigten HSCG+S lag hingegen während der Transaktivierung keine Regulation der miR-122-Expression vor. Zusätzlich konnte in HSCG im Gegensatz zu hochreinen HSCG+S eine positive Korrelation der miR-122-Expression mit der Expression von Hepatozyten-Markern festgestellt werden. **Schlussfolgerung:** Unsere Ergebnisse legen folglich nahe, dass die beschriebene Runterregulation von miR-122 in aktivierten HSCG ein Artefakt der initialen Hepatozyten-Kontamination darstellt und dass während der Hepatofibrogenese, entgegen veröffentlichter Arbeiten, miR-122 nicht in der HSC-Aktivierung reguliert ist.

1.13 Differentiation and effects of TGF-β1 and IL-3 signaling on primary murine endoglin deficient mast cells

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Background: Mast cells (MCs) are components of the innate immune system and are recruited to the inflamed and fibrotic liver [1]. Mast cell effects are in part based on the secretion of mitogenic and pro-fibrotic cytokines (e. g. TGF-β1) leading to activation and proliferation of fibroblasts [2]. Nevertheless, MCs themselves are targets of TGF-β1 and do express the accessory TGF-β receptor endoglin with yet unknown function in MCs [3]. **Material and Methods:** Endoglin deficient mast cells were generated by crossing mast cell specific Cre-expressing mice [4] with mice carrying floxed Endoglin alleles [5]. Primary MCs were isolated and expanded (peritoneal mast cells; PMC) or differentiated (bone marrow derived mast cells; BMMC) and analyzed by flow cytometry, cell counting and Western blot analysis [6]. **Results:** FACS analysis revealed that wildtype and Endoglin deficient PMC and BMMC achieved full maturity as demonstrated by detection of the surface markers c-kit and FcεR1α. However, PMC expansion from MC progenitors deficient in Endoglin was delayed. TGF-β1 reduced proliferation in the presence or absence of IL-3 without marked differences between the genotypes, whereas the amount of dead (apoptotic) cells in the presence of TGF-β1 is reduced in BMMC that lack Endoglin. Finally, we found that Endoglin affects the secretion of tryptase and granzyme B (gzmB) in the process of degranulation. **Conclusions:** These results imply a function of endoglin in PMC differentiation and MC apoptosis. During degranulation, Endoglin affects the secretion of gzmB and tryptase from its cytoplasmic granules. This activity plays a critical role in the process of fibrosis. **References:** [1] Jones H et al. Inhibition of mast cell-secreted histamine decreases biliary proliferation and fibrosis in primary sclerosing cholangitis Mdr2(-/-) mice. Hepatology 2016. doi: 10.1002/hep.28704 [Epub ahead of print]. [2] Evans RA et al. TGF-β1-mediated fibroblast-myofibroblast terminal differentiation-the role of Smad proteins. Exp Cell Res 2003;282:90 – 100. [3] Gebhardt T et al. Growth, phenotype, and function of human intestinal mast cells are tightly regulated by transforming growth factor β1. Gut 2005;54:928 – 34. [4] Scholten J et al. Mast cell-specific Cre/loxP-mediated recombination in vivo. Transgenic Res 2008;17:307 – 15. [5] Allinson KR et al. Generation of a floxed allele of the mouse endoglin gene. Genesis 2007;45:391 – 95: [6] Meurer SK et al. Isolation of mature (peritoneum-derived) mast cells and immature (bone marrow-derived) mast cell precursors from mice. PLoS ONE 2016;11(6):e0158104.

1.14 Hepatic stellate cells down-regulate pro-apoptotic effector abundance and secretion in mast cells

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Background: Fibrosis and its resolution depend on hepatic stellate cell (HSC) activation and apoptosis [1]. Mast cells (MCs) are recruited to the

liver during fibrosis and they do express potent pro-apoptotic proteins granzyme B (gzmB) and cleaved caspase 3 which are both functionally linked [2, 3]. **Methods and Results:** Co-culture of HSC and MCs (L138.8A) leads to an increase in gzmB and decrease in cleaved caspase 3 abundance. This modulation in protein quantities is accompanied by reduced STAT5 and p42/p44 activation. In line, the treatment of MC (L138.8A) with TGF-β1 leads to a decrease of cellular activated gzmB and cleaved caspase 3. This coincides with a reduced activation of STAT5 and p42/p44 which is observed in L138.8A and in primary isolated peritoneal mast cells (PMC) [4]. L138.8A which are challenged with IgE and antigen, secrete gzmB and cleaved caspase 3 in an ERK-dependent manner. Secretion of both proteases is reduced in the presence of TGF-β1, an effect which can be reverted by the ALK5 inhibitor SB431542 in case of cleaved caspase 3. **Conclusions:** These results suggest that TGF-β1 that is released by activated HSC in co-culture leads to a reduction in STAT5 and ERK activity causing reduced secretion of active cleaved caspase 3. **References:** [1] Puche JE, Saiman Y, Friedman SL. Hepatic stellate cells and liver fibrosis. Compr Physiol 2013;3:1473 – 92. [2] Pardo J, Wallich R, Ebnet K, Iden S, Zentgraf H, Martin P, Ekiciler A, Prins A, Müllbacher A, Huber M, Simon MM. Granzyme B is expressed in mouse mast cells in vivo and in vitro and causes delayed cell death independent of perforin. Cell Death Differ 2007;14:1768 – 79. [3] Zorn CN, Pardo J, Martin P, Kuhny M, Simon MM, Huber M. Secretory lysosomes of mouse mast cells store and exocytose active caspase-3 in a strictly granzyme B dependent manner. Eur J Immunol 2016;43:3209 – 18. [4] Meurer SK, Neß M, Weiskirchen S, Kim P, Tag CG, Kauffmann M, Huber M, Weiskirchen R. Isolation of mature (peritoneum-derived) mast cells and immature (bone marrow-derived) mast cell precursors from mice. PLoS ONE 2016;11(6): e0158104.

1.15 Primary peritoneal mast cells (PMC) reduce the fibrogenic response of hepatic stellate cells while inducing caspase 3 cleavage and activity

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Background: Mast cells (MCs) are recruited to the liver during fibrosis and induce detachment- dependent apoptosis in mouse embryonic fibroblasts [1]. We have previously shown that co-culture of a murine MC line (L138.8A) with the murine hepatic stellate cell line HSC/Col-GFP [2] leads to a strong reduction of the fibrotic response in HSC by affecting several aspects of TGF-β signaling [3]. **Methods and Results:** We show here that L138.8A cells are able to reduce fibrogenic responses in primary rat HSC as well. Primary murine Peritoneum-derived MC were isolated and cultured as described before [4]. Similar effects on attenuation of pro-fibrogenic responses were observed in HSC/Col-GFP when co-cultured with primary peritoneal MC (PMC). In addition the expression of cyclin A is reduced pointing towards a lower cell cycle/proliferation rate. Moreover under optimal conditions (IL-3 and SCF supplementation), PMC induce caspase 3 cleavage which is accompanied by a reduction in Poly (ADP-ribose) polymerase (PARP) abundance. These effects are paralleled by a strong increase in STAT5 activity. **Conclusions:** Primary mouse PMC reduce the fibrogenic and proliferative response while inducing the apoptotic response in HSC. Our study suggests that targeting the activity of PMC in hepatic fibrogenesis may provide therapeutic benefits during phases of liver injury. **References:** [1] Pardo J, Wallich R, Ebnet K, Iden S, Zentgraf H, Martin P, Ekiciler A, Prins A, Müllbacher A, Huber M, Simon MM. (2007) Granzyme B is expressed in mouse mast cells in vivo and in vitro and causes delayed cell death independent of perforin. Cell Death Differ. 14:1768 – 79. [2] Meurer SK, Alsamman M, Sahin H, Wasmuth HE, Kisseleva T, Brenner DA, Trautwein C, Weiskirchen R, Scholten D. (2013) Overexpression of endoglin modulates TGF-β1-signalling pathways in a novel immortalized mouse hepatic stellate cell line. PLoS ONE 8 (2):e56116. [3] Meurer SK, Neß M, Weiskirchen R. (2015) Mast cells inhibit activation and profibrogenic activities of hepatic stellate cells. Z Gastroenterol, 53:1540. [4] Meurer SK, Neß M, Weiskirchen S, Kim P, Tag CG, Kauffmann M, Huber M, Weiskirchen R. (2016) Isolation of mature (peritoneum-derived) mast cells and immature (bone marrow-derived) mast cell precursors from mice. PLoS ONE 11(6): e0158104.

1.16 Lipocalin 2 (LCN2)-deficient mice are more prone to hepatic steatosis: LCN2 and mitochondrial and peroxisomal integrity

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Background: Lipocalin-2 (LCN2) or neutrophil gelatinase-associated lipocalin (NGAL) is a small secreted adipokine associated with transport of small hydrophobic molecules [1]. In the liver, it limits bacterial growth and modulates the inflammatory response by acting as a “help me” signal attracting circulating blood cells into the tissue [2]. We have previously demonstrated that LCN2 is involved in control of hepatic fat metabolism by regulating the expression of the intracellular lipid droplet protein PLIN5/O’X’PAT [3]. **Methods:** We here extended our work and performed a comparative proteome profiling of wild type and Lcn2-deficient mice that were fed either with a standard chow or a methionine- and choline-deficient diet. Histological scoring of steatosis was adapted from guidelines used in the scoring of non-alcoholic fatty liver disease in humans. Alterations in protein expression were confirmed by Western blot and real-time PCR analysis. Mitochondrial and peroxisomal integrity was evaluated by a large variety of methods. To estimate the biological significance of LCN2 in lipid homeostasis, we compared tissue sections of Lcn2-deficient mice or WT mice fed an MCD diet by Matrix-Assisted Laser Desorption/Ionization Time-Of-Flight (MALDI-TOF) mass spectrometry imaging. **Results:** Gene ontology enrichment analysis performed with the web-based application Gene Ontology enRIchment anaLysis and visuaLizAtion (GOrilla) identified enriched GO terms that are associated with mitochondrial function and carboxylic acid metabolic processes. Comparative measurements of the mitochondrial iron pool, membrane potential, intracellular lipid peroxidation, and peroxisome staining confirmed that the presence of LCN2 impacts mitochondrial function and peroxisome quantities in primary hepatocytes. In regard to fat analysis, significant changes to m/z signal intensities for various sphingomyelins, triglycerides, and glycerophospholipid species were identified. In the liver, the loss of Lcn2 was associated with an increase in two arachidonic acid containing glycerophospholipids suggesting that LCN2 is critically involved in polyunsaturated fatty acid metabolism. **Conclusions:** In summary, our data shows that LCN2 is a versatile serum protein that impacts protein and fat homeostasis and further controls the integrity and function of mitochondria and peroxisomes. **References:** [1] Asimakopoulou A, Weiskirchen R. Lipocalin 2 in the pathogenesis of fatty liver disease and non-alcoholic steatohepatitis. Clin Lipidol. 2015;10:47 – 67. [2] Asimakopoulou A, Borkham-Kamphorst E, Tacke F, Weiskirchen R. Lipocalin-2 (NGAL/LCN2), a "help-me" signal in organ inflammation. Hepatology 2016;63:669 – 71. [3] Asimakopoulou A, Borkham-Kamphorst E, Henning M, Yagmur E, Gassler N, Liedtke C, Berger T, Mak TW, Weiskirchen R. Lipocalin-2 (LCN2) regulates PLIN5 expression and intracellular lipid droplet formation in the liver. Biochim Biophys Acta 2014;1842:1513 – 24.

1.17 The CYR61/CTGF/NOV (CCN) family members induce endoplasmic reticulum stress and unfolded protein response

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Background: The endoplasmic reticulum (ER) is the site of synthesis and folding of secreted membrane-bound and diverse organelle-targeted proteins. This process requires several factors, including ATP, Ca2+ and an oxidizing environment for proper disulphide-bond formation. The ER is sensitive to stress factors that perturb cellular energy levels, cellular

redox state, or intracellular Ca2+ content. As a consequence, these alterations reduce the protein folding capacity of the ER leading to accumulation and aggregation of unfolded proteins ending an orchestrated response which is known as unfolded protein response (UPR). We and others have shown that matricellular proteins of the CCN (CYR61, CTGF, NOV) family play essential roles in extracellular matrix signaling and turnover [1 – 3]. They share a similar architecture and contain a high content of cysteine residues. Previously, we have shown that large quantities of CCN1/CYR61 induce reactive oxygen species formation in portal myofibroblasts and ER stress in hepatic stellate cells (HSC) [1, 2]. **Methods:** We here used adenoviral-mediated gene transfer of CCN2/CTGF, CCN3/NOV and CCN4/WISP-1 in primary HSC and hepatocytes. Expression analysis of ER-stress related genes (Chop, Ire1α, Perk, Bip, Grp94, Dnajc3, Dnajb9, Trib) and splicing of Xbp-1 was done by qRT-PCR. Cellular senescence and apoptosis was measured by β-galactosidase and TUNEL assay. In vitro and in vivo expression of adenoviral constructs was done as described elsewhere [3]. **Results:** We found that similar to CCN1, the three other members of the CCN protein family induce splicing of Xbp-1 (L) to Xbp-1 (S) initiating ER stress and UPR when expressed in high quantities. In addition, typical marker genes of ER-stress and UPR (BIP, IRE1α, PDI, Chop) are induced in cells that express high quantities of CCNs. This capacity provides the basis for a physiological self-limiting process that restricts hepatic fibrogenesis. Moreover, intravenous injections of high quantities of Ad-CCNs via the tail vein were suitable to increase Bip and Chop mRNA expression and to decrease splicing of Xbp-1 (L). **Conclusions:** The observed CCN-induced UPR is relevant in wound healing responses and essential for hepatic tissue repair following liver injury. We suggest that the targeted transfer of adenoviral vectors triggering high expression of CCN proteins to profibrogenic cells is therapeutically effective to limit hepatic fibrosis. **References:** [1] Borkham-Kamphorst E, Schaffrath C, Van de Leur E, Haas U, Tihaa L, Meurer SK, Nevzorova YA, Liedtke C, Weiskirchen R. The anti-fibrotic effects of CCN1/CYR61 in primary portal myofibroblasts are mediated through induction of reactive oxygen species resulting in cellular senescence, apoptosis and attenuated TGF-β signaling. Biochim Biophys Acta 2014;1843: 902 – 14. [2] Borkham-Kamphorst E, Steffen BT, Van de Leur E, Haas U, Tihaa L, Friedman SL, Weiskirchen R. CCN1/CYR61 overexpression in hepatic stellate cells induces ER stress-related apoptosis. Cell Signal 2016;28:34 – 42. [3] Borkham-Kamphorst E, Huss S, Van de Leur E, Haas U, Weiskirchen R. Adenoviral CCN3/NOV gene transfer fails to mitigate liver fibrosis in an experimental bile duct ligation model because of hepatocyte apoptosis. Liver Int. 2012;32:1342 – 53.

1.18 Untersuchung von Lipocalin 2 und Perilipin 5 in der Fruktose-induzierten Nichtalkoholischen Fettlebererkrankung

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Hintergrund: Von den Lebererkrankungen zählt die Nichtalkoholische Fettlebererkrankung (NAFLD) zu den häufigsten Lebererkrankungen. Gleichzeitig fördert eine gesteigerte Aufnahme von Fruktose die Entstehung einer Fettleber [1]. Die Pathogenese der NAFLD ist bislang noch unzureichend bekannt und Therapiemöglichkeiten begrenzt [2]. Lipocalin 2 (LCN2) ist ein Transportprotein, das Fette binden und dadurch die Mobilisierung von Fettreserven regulieren kann [3]. Eine Hochregulation von LCN2 ist als Antwort auf akute oder chronische Schädigung der Leber zu beobachten, die mit dem Grad des Entzündungsgeschehens korreliert [4, 5]. LCN2 reguliert Perilipin 5 (PLIN5/OXPAT), das Speicherung und Ausschüttung von Triacylglyceriden aus Lipidtröpfchen reguliert und vor Lipotoxizität schützt [6]. In Lebern mit Steatose die Expression von PLIN5 gesteigert [6]. **Ziele:** Ziel der Studie ist es, die Funktion von LCN2 und PLIN5 in der Entstehung und Progression der NAFLD zu ergründen. **Methodik:** Wildtyp und LCN2-defizienten Mäusen wurde über einen Zeitraum von 4 bzw. 8 Wochen hohe Mengen von Fruktose über die Nahrung oder Wasser zugeführt. Anschließend wurden die hepatische Expression von LCN2 und PLIN5 mittels quantitative real time PCR und Western Blot untersucht. Die Schädigung und die Verfettung der Leber wurde in histologischen Schnitte mittels Hematoxylin/Eosin und Oil Red Färbung dargestellt. **Ergebnisse/Schlussfolgerung:** Die Expression von LCN2 und PLIN5 steht in engem regulatorischen Bezug. Durch die übermäßige Aufnahme von Fruktose in Wasser oder Futter wird eine Leberverfettung induziert, die sich in histologischen Färbungen gut darstellen läst. Der dauerhafte Konsum von Fruktose erzeugt in der Leber eine simultane Expressionssteigerung von LCN2 und PLIN5. Weiterhin zeigen sich geschlechtsspezifische Unterschiede in der Pathogenese der durch Fruktose induzierten Leberschädigung. **Referenzen:** [1] Perry et al. Nature 2014;510:84 – 91. [2] Alwahsh et al. World J Gastroenterol. 2014;20: 1807 – 21. [3] Zhao et al. J Biol Chem. 2014;289:5960 – 9. [4] Borkham-Kamphorst et al. Biochim Biophys Acta 2013;1832:660 – 73. [5] Asimakopoulou et al. Biochim Biophys Acta 2014;1842:1513 – 24. [6] Wang et al. Hepatology 2015;61:870 – 82.

1.19 Effects of radiation and/or tumor necrosis factor alpha on cell damage in a healthy liver: a role for PECAM-1

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The liver is considered to be radiosensitive; however, the mechanisms of radiation-induced liver damage are poorly understood. Platelet endothelial cell adhesion molecule 1 (PECAM-1/CD31) is an adhesion molecule and expressed mainly in blood cells and endothelial cells. Its expression is decreased during inflammatory processes. Tumor necrosis factor (TNF)-alpha, which is induced by radiation, is known to downregulate PECAM-1. The aim of the current study was to investigate if combined treatment with TNF-alpha and irradiation would enhance liver damage through regulation of the PECAM-1 signaling pathway. This was studied in-vivo in mouse models of single-dose selective liver irradiation w/wo TNF-alpha administered intraperitoneally shortly before irradiation. The mice were sacrificed at different time points, serum and tissues were collected for further analyses. RNA- and protein analyses were performed by RT-PCR and Western blotting, respectively. Both irradiation and TNF-alpha administration alone induced elevated aspartate aminotransferase (AST)-levels (hepatic damage) in serum, compared to sham-irradiated mice (control). This hepatic damage was further enhanced in mice that received combined treatment with irradiation and TNF-alpha. In parallel to hepatic damage, a time-dependent decrease in the expression level of hepatic PECAM-1 was found in mice that received each single irradiation or TNF-alpha treatment. The administration of irradiation together with TNF-alpha showed additional decline in the expression of PECAM-1. In contrast, increased expression of hepatic lipocalin-2 (LCN-2), an acute phase protein, was detected at mRNA and protein levels after irradiation or TNF-alpha treatment. The level of LCN-2 was further increased in mice that received combined treatment with TNF-alpha and irradiation, compared to irradiation or TNF-alpha alone. This induction seems to be mediated by the activation of the signal transducer and activator of transcription (STAT)-3 signaling pathway. In order to study the role of PECAM-1 in hepatic damage, the liver of both wild-type (WT) and PECAM-1 knock-out (KO)-mice were selectively irradiated (25 Gy). PECAM-1 KO mice showed higher liver damage in parallel to increased LCN-2 expression compared to WT-mice at RNA and protein levels. By means of Western blotting, an increased level of cell death-related proteins (SOD-1, BAX) was observed after irradiation in both WT- and PECAM-1 KO mice. However, the level of Cyt-C was reduced only in PECAM-1 KO mice after irradiation. Our study shows a synergistic effect of radiation and TNF-alpha on hepatic cell-damage, probably through regulation of PECAM-1. Our results may help to develop protective strategies to reduce radiation-induced defects in normal liver tissue, as well as strategies, which may increase the effects of radiation on tumor tissue.

1.20 Expression of the oncofetal marker Nope in the regenerating adult murine liver after disruption of interhepatocytic gap junctions via bile duct ligation

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Background: Neighbor of Punc E11 (Nope) is strongly expressed in fetal and adult hepatic stem/progenitor cells and in hepatocellular carcinoma but not in terminally differentiated and normally polarized hepatocytes. We here investigated the expression pattern of the oncofetal marker Nope in adult mice after bile duct ligation leading to disruption of interhepatocytic gap junctions. **Methods:** Liver tissue was extracted from adult C57Bl6 mice 24 hours up to 4 weeks after bile duct ligation

(BDL). Liver tissue was tested for expression levels of Nope via quantitative RT-PCR and for Nope and the gap junction protein Connexin 26 via Western blotting. Costainings were performed for Nope in combination with Connexin 26, CK19 (biliary), E-cadherin (epithelial) or the canalicular marker dipeptidylpeptidase (DPP) IV. **Results:** Bile duct ligation leads to a significantly increasing expression level of Nope (after 1 week 87-fold vs. adult liver, $p < 0.0001$, after 4 weeks 676-fold vs. adult liver, $p < 0.001$). In Western blot, this high expression level of Nope after BDL can be confirmed, while the expression level of Connexin 26 is markedly downregulated. In immunohistochemistry, almost all of the hepatocytes stain positive for Nope at later stages after BDL. Costainings with E-cadherin and DPPIV demonstrate a regular sinusoidal expression pattern of Nope on hepatocytes, but no expression on CK19-positive cholangiocytes. Costainings with Connexin 26, that is equally distributed in normal adult liver, reveal a substantial overlap and staining for Connexin 26 is almost restricted to Nope-positive hepatocytes. **Conclusion:** We here report the expression of the oncofetal marker Nope on adult hepatocytes after disruption of interhepatocytic gap junctions via BDL, while the expression of Connexin 26 is markedly downregulated and mainly limited to Nope-positive hepatocytes. With regard to its structural similarity to axonal guidance receptors, the induction of Nope might be a compensatory mechanism of "disorientated" hepatocytes to regain hepatocellular polarization within the regenerating parenchymal liver network.

1.21 Fibrosis in adipose tissue is correlated to liver injury in NAFLD

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Background and Aims: Fibrosis occurs in different organs and is associated with damage. In non-alcoholic fatty liver disease (NAFLD), liver fibrosis is also a sign for progression. Adipose tissue seems to play an important role in the progression of NAFLD, but the contribution of adipose tissue fibrosis is unclear. The aim of our study was: (i) to check if adipose tissue in obesity exhibits fibrosis, (ii) if fibrosis in adipose tissue correlates with liver damage and fibrosis and (iii) autophagy in adipose tissue is associated with fibrosis in adipose tissue. **Methods:** Blood, visceral adipose and liver tissue samples were obtained from 62 (mean age: 43+/-10 y. w:45/m:17) morbidly obese patients undergoing bariatric surgery. Fibrosis was assessed by Sirius Red staining in adipose and liver tissue. mRNA expression of genes related to fibrosis (collagen) and autophagy (ATG5, LC3, Beclin) were measured in adipose and liver tissue by qrt-PCR. ATG 5 staining in adipose tissue was performed by immunohistochemistry and quantitatively analyzed. Blood samples were analyzed for routine parameters and surrogate markers of apoptosis and cell death (M30, M65) by ELISA. **Results:** Increased fibrosis was observed by Sirius red and collagen expression was significantly upregulated ($p < 0.0001$) in adipose tissue of morbidly obese patients compared to controls. mRNA levels of LC3 and Beclin were significantly increased in adipose tissue of obese patients compared to controls (LC3 $p < 0.02$; Beclin $p < 0.05$). In addition the amount of ATG5-positive cells in adipose tissue was significantly correlated with fibrosis in adipose tissue. Significant correlations were also found between adipose tissue fibrosis and serum M30 as well as expression of autophagy related genes in adipose tissue. Autophagy related gene expression in liver and adipose tissue was also correlated. **Conclusions:** Morbid obesity leads to fibrosis in adipose tissue, which is associated to elevated expression of autophagy related genes. Moreover, autophagy in adipose tissue and liver tissue are correlated, implicating a possible common signalling axis leading to increased autophagic processes in different tissues. The underlying mechanisms need further characterization.

1.22 Generation and functional analyses of hepatocyte-specific type I interleukin-1 receptor (IL-1RI) knockout mice

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Background: IL-1-type cytokines including IL-1α, IL-1β and IL-1Ra are among the most potent molecules of the innate immune system and exert their biological function through the ubiquitously expressed IL-1RI. The role of IL-1RI has been studied in myeloid-derived cells, fibroblast and endothelium, however its role in hepatocytes during acute and chronic liver disease remains to be determined. **Methods:** Using the Cre/loxP system, we generated a mouse that lacks IL-1RI specifically in hepatocytes. Hepatocyte-specific deletion of IL-1RI was achieved by crossing IL-1RIflox/flox mice, in which exon 5 of the Il1r1 gene is flanked by loxP sites, with mice expressing Cre-recombinase under an albumin promoter generating new albumin-cre:IL-1RIflox/flox (IL-1RIHep-/-) mice. **Results:** IL-1RIHep-/- mice appeared healthy, had normal ALT and AST levels and normal liver histology at 6 months of age. No metabolic or liver-specific phenotypes were observed at this age. IL-1RIHep-/- mice exhibited a significant > 99% reduction of Il1r1 mRNA expression in primary hepatocytes compared to wild type (wt) hepatocytes. Expression levels of Il1a and Il1b mRNA were also significantly reduced (14.7% and 9.1%). To examine the functional effect of IL-1RI deletion, in vitro stimulation of IL-1RIHep-/- hepatocytes with recombinant mouse IL-1α protein (10 ng/ml) was performed, which did not lead to an upregulation of Il1r1 mRNA in IL-1RIHep-/- hepatocytes, whereas wt hepatocytes exhibited a 1.8-fold induction of Il1r1 mRNA. Also the mRNA expression of the target genes Il1b, Il6 and Ccl2 was induced only in wt hepatocytes following stimulation with recombinant mouse IL-1α. However, in response to LPS (10 µg/ml) we detected an upregulated expression of Il1a, Il1b, Il6 and Ccl2 mRNA irrespective of the genotype, although the absolute expression levels in IL-1RIHep-/- hepatocytes were below the levels in the wt. Il1r1 mRNA was again only 1.8-fold upregulated in wt hepatocytes. **Conclusion:** This novel IL-1RIHep-/- mouse model exhibits a functional deletion with loss of signaling through IL1-RI in hepatocytes and allows to further investigate this signaling pathway in acute and chronic liver disease.

1.23 Genetic analysis of spontaneous (non-toxic) liver fibrosis in a congenic mouse intercross

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Background: Mutations in the ABCB4 (ATP-binding casette, subfamily B, member 4) gene cause cholestatic liver diseases including progressive intrahepatic familial cholestasis (PFIC). Modifier genes have yet to be identified systematically. In this study we used the Abcb4 (Mdr2) knockout (-/-) mouse model. The deficiency of the hepatobiliary phosphatidylcholine floppase leads to chronic cholestasis, liver injury and fibrosis. As different mouse strains show varying fibrosis susceptibility, we applied a systematic approach to elucidate the genetic control of liver fibrosis in an experimental cross of ABCB4 deficient congenic strains. **Methods:** The Abcb4 knockout was transferred from the fibrosis-resistant FVB-Abcb4-/- strain to the susceptible BALB/cJ strain by repeated backcrossing. To identify genetic modifiers that contribute to the fibrosis susceptibility linked to ABCB4 deficiency, we crossed these two congenic strains to generate an F2 intercross population. By quantitative trait locus (QTL) analysis differences in disease progression were mapped to polymorphic genetic regions across the whole genome. Single and two-dimensional QTL scans were applied to identify modifiers and pairwise gene interactions. **Results:** Compared to FVB-Abcb4-/- mice, the BALB-Abcb4-/- mice progress to higher fibrosis stages. The heterogenic F2 population shows marked phenotypic variation. Whereas single modifiers demonstrate minor effects, gene-gene interaction scans identified a significant interaction of two QTLs on chromosomes 4 and 17. Underlying these loci we identified the genes Abcg5, Abcg8 (ATP binding cassette subfamily G members 5 and 8) and sterol carrier protein 2 (Scp2) that are functionally related with hepatobiliary cholesterol homeostasis and resemble

creedal modifer genes. **Conclusions:** The congenic Balb-Abcb4 knockout mouse allows the genomic exploration of a spontaneous, non-toxic disease model of a human gene defect. The experimental cross of the two genetic backgrounds with distinct fibrosis susceptibility enables the identification of Abcb4-dependent modifiers of cholestatic liver diseases.

1.24 Hepatic Nrf2 overexpression inhibits the deleterious effects induced by c-met deficiency in the progression of NASH

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Background: We recently showed that hepatocyte-specific c-met deficiency accelerates the progression of non-alcoholic steatohepatitis in experimental murine models resulting in increased hepatic lipotoxicity, augmented production of reactive oxygen species and accelerated development of fibrosis. The aim of this study focuses on the elucidation of the underlying cellular mechanisms driven by Nrf2 over-activation in hepatocytes lacking c-met receptor characterized by a severe unbalance between pro-oxidant and anti-oxidant functions. **Methods:** For this purpose, we generated double knockout mice lacking simultaneously c-met and keap1 genes employing the flox/cre technology under the control of the albumin promoter. Control mice (c-metfx/fx), single c-met knockouts (c-metΔhepa) and double c-met/keap1 knockouts (met/keap1Δhepa) were then fed a methionine-choline deficient (MCD) diet for 4 weeks in order to reproduce the features of NASH. Serum and liver samples were collected for biochemical, RNA and protein expression analyses. **Results:** Double mutants displayed an increased liver mass as compared to the other experimental groups, but present less triglycerides accumulation. The marked increase of oxidative stress observed in c-metΔhepa was restored in the double mutants as assessed by 4-HNE immunostaining and by the expression of genes involved in the control of redox homeostasis, such as Cyp2e1 and NOX2. Accordingly, the number of TUNEL positive cells was also dramatically decreased in met/keap1Δhepa. Double ko mice also presented a reduced amount of liver infiltrating cells – neutrophils and resident macrophages (CD11b+/F4 – 80+) – as emerged by flow cytometry analysis of intrahepatic lymphocytes. These data were further supported by RT-PCR analyses indicating a decrease of the proinflammatory cytokine MCP1 in the double knockouts as compared to the other groups. Similarly, the worsening of fibrosis progression observed in c-metΔhepa livers was efficiently reduced at the levels of controls as indicated by Sirius Red staining and expression of pro-fibrotic mediators such as TGFb1 and Col1a1. **Conclusion:** Genetic activation of the antioxidant transcription factor Nrf2 improves liver damage and repair in c-met deficient mice mainly through restoring a balance in the cellular redox homeostasis. This observation arouses further considerations for the development of novel therapeutic strategies.

1.25 Hypoxia causes hepatic stellate cells activation in the absence of CUX1

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Background and Aim: CUX1 (CUTL1) is a transcription factor belonging to homeobox proteins. It is responsible of driving the transcription of genes deputed to many cellular functions like proliferation, differentiation and cell death. It has been shown that its role can change and drive tumorigenesis. Up to now, its role is unknown in hepatic stellate cells. We focused on CUX1 activity in hepatic stellate cells undergoing hypoxic stress. **Methods:** LX-2 cells were treated with 100 ng/ml CoCl2 or kept at 37 ° C at lox oxygen (< 0.5%). Expression of hypoxia markers and activation markers was performed by RT-qPCR. Western blotting was performed to analyze the protein level of CUX1 and HIF-1alpha. **Results:** LX-2 cells treated for 6 hours with CoCl2 or low oxygen showed an overexpression or a restoration of COL1A1 and ACTA2 after knock down of CUX1. Additionally, CDKN1A, CDKN1B, VEGFA and HIF1alpha were up-regulated in LX.2 cells previously transfected with siCUX1. Protein level of CUX1 was significantly down-regulated, whereas HIF1alpha protein was strongly up-regulated by hypoxia condition. **Conclusion:** CUX1 controls the activation of hepatic stellate cells. Its knock down promotes the hypoxia response. CUX1 could represent a key factor for controlling liver fibrogenesis. Its role in a liver fibrosis scenario needs to be further investigated.

1.26 IL-1β induced activation of the p38MAPK/MK2 pathway in hepatocytes and macrophages: a mathematical model of cell-type-specific signal transduction

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In hepatocytes and in macrophages, which mediate the inflammatory response in the liver upon challenge, the activation of the p38MAPK/MK2 pathway by IL-1β is important for the control of acute phase response as well as for liver regeneration upon damage. Little is known about key characteristics of this pathway in terms of concentration-dependent signal propagation and cell-type specific responses. Based on a mathematical model the present study provides evidence that signal transduction from IL-1β via p38MAPK to MK2 is characterized by a strong signal amplification. Quantification of the intracellular phosphorylation level of the residues Thr180 and Tyr182 of p38MAPK and Thr200 of MK2 reveals that in primary murine hepatocytes at maximum 11.3% of p38MAPK molecules and 36.5% of MK2 molecules are activated in IL-1β signaling. Furthermore, in silico analyses demonstrate that the kinase activity of p38MAPK determines the signal amplitude while phosphatase activity affects both signal amplitude and signal duration. In contrast to this in bone marrow derived macrophages at maximum only 4.5% of p38MAPK molecules and 17,2% of MK2 molecules are activated upon treatment with IL-1β, whereas quantification of p38MAPK and MK2 total protein reveals that the intracellular concentration in macrophages is approximately three times higher than in hepatocytes. We conclude that even with a lower percentage of activated p38MAPK and MK2 macrophages display comparable or even higher phosphorylation levels than hepatocytes. Hence, the mathematical model of this study reveals cell-type-specific differences concerning the response towards the treatment with IL-1β.

1.27 Inflammation and Fibrosis in the Livers of TNFR1 / Mdr2ko Mice

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Hepatocellular carcinoma (HCC) develops in chronically inflamed livers with advanced fibrosis in 90% of all cases. The multidrug resistance protein 2 knockout (Mdr2ko) mice are a well-established model for chronic hepatitis and inflammation-associated HCC. Tumor necrosis factor receptor-1 (TNFR1) mediated signaling is known to induce epithelial cell death, inflammation, and fibrosis. This study aims to analyze whether an additional knockout of TNFR1 in the Mdr2ko mouse model would reduce hepatic inflammation and fibrosis, which could consequently delay HCC development. Tissue injury was assessed by plasma analysis of ALT and ALP levels. Hepatic immune phenotyping was determined by FACS analysis. Inflammation and fibrosis were evaluated by histological analysis of H&E stained liver slices. ECM deposition was analyzed by quantifying the hepatic hydroxyproline content and Sirius Red staining. Real time RT PCR was applied to analyze hepatic expression levels of targets involved in inflammation (IL-1β, TNFα), matrix remodeling (Collagen, MMPs, TIMPs, α-sma), tumor development (AFP, A20, OPN). ALT and ALP levels were increases in TNFR1/Mdr2ko mice, which is indicative of more severe tissue damage. TNFR1/Mdr2ko mice showed significantly increased hepatic hydroxyproline contents. Real time RT-

PCR showed elevated expression levels of genes involved in fibrosis, inflammasome activity, necroptosis and possibly malignant transformation. The absence of TNFR1 mediated signaling does not improve the pathological phenotype of Mdr2ko mice. It instead exacerbated tissue damage possibly through alternative forms of cell death, which results in an increased fibrotic response. Long-term studies will have to show whether this will affect the tumor incidence in the Mdr2ko mouse model.

1.28 Influence of hepatocytes on macrophage differentiation

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The liver comprises the body's largest pool of macrophages constituting approximately 80% of all sessile tissue macrophages of the body. Thereby macrophages have a remarkable plasticity since their differentiation and function is continuously adapted to the microenvironment, which among others is defined by direct and indirect intercellular communication including communication via cell-cell contact and paracrine acting mediators. The present study investigates the impact of the intercellular communication between macrophages and hepatocytes on macrophage differentiation and function and involves a trans-well co-culture model in which cells are separated by a membrane that allows mediator exchange but not direct cellular interaction. The data indicate that co-cultivation with hepatocytes leads to a special phenotype of BMDM. This population of macrophages is characterized by the up-regulation of Gr-1, CD 163, CD 206, MHC class II and TLR4 as well as an increased expression of Arginase1, Stabilin1, CD 16, CD 32 and iNOS. These changes were accompanied by changes of the inflammatory response of BMDM towards LPS treatment, since co-culture with hepatocytes resulted in a significant increase of IL-10, IFNβ and Arginase1 expression and a reduced and/or less sustained expression of the cytokines TNFα, IL-6 and IL-12. Evidence is provided that hepatocytes and macrophages maintain a complex intercellular communication network resulting in a macrophage phenotype that is characterized by expression of markers indicative for wound-healing type sessile tissue macrophages displaying a predominant anti-inflammatory cytokine release upon activation with LPS characterized by an increase of the IL-10/IL-12 ratio.

1.29 Novel rat model of repetitive portal venous embolization mimicking human non-cirrhotic idiopathic portal hypertension

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Background: Non-cirrhotic idiopathic portal hypertension (NCIPH) is characterized by splenomegaly, anemia and portal hypertension, while liver function is preserved. However, no animal models have been established yet. This study assessed a rat model of NCIPH and characterized the hemodynamics, and compared it to human NCIPH. **Methods:** Portal pressure (PP) was measured invasively and coloured microspheres were injected in the ileocecal vein in rats. This procedure was performed weekly for 3 weeks (weekly embolization). Rats without and with single embolization served as controls. After four weeks (one week after last embolization), hemodynamics were investigated, hepatic fibrosis and accumulation of myofibroblasts were analysed. General characteristics, laboratory analyses and liver histology were collected in patients with NCIPH. **Results:** Weekly embolization induced a hyperdynamic circulation, with increased PP. The mesenteric flow and hepatic hydroxyproline content was significantly higher in weekly embolized compared to single embolized rats (mesenteric flow +54.1%, hydroxyproline +41.7%). Mesenteric blood flow and shunt volumes increased, whereas splanchnic vascular resistance was decreased in the weekly embolization group. Fibrotic markers αSMA and Desmin were upregulated in weekly embolized rats. **Discussion:** This study establishes a model using repetitive embolization via portal veins, comparable with human NCIPH and may serve to test new therapies.

1.30 Role of NLRP3 inflammasome activation during cholestatic liver injury

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Accumulation of toxic bile salts in hepatocytes leads to hepatic toxicity and injury by cell death. Apoptotic and pyroptotic cell death is a consequence of caspase-8 or caspase-1 activation and is mediated by the NLRP3 inflammasome. The activation of the NLRP3 inflammasome results in liver inflammation, fibrosis and hepatocyte pyroptosis in mice. Thus we investigate what the role of the NLRP3 inflammasome activation is during cholestatic liver injury. To evaluate the role of the NLRP3 inflammasome we used the bile duct ligation (BDL) model (as established by Weiskirchen et al.) in WT and NLRP3-/-. After performing BDL we waited 2 days (acute model) or 28 d (chronic model) and sacrificed the mice. Inflammation, fibrosis and cell death were evaluated with qPCR, IHC, IF and western blot. After 2 d of BDL we can see that NLRP3-/- mice have elevated serum transaminases compared to WT mice. In addition, hepatic inflammation, as shown by mRNA levels of TNFα, IL-1b, MCP-1 and influx of Ly6G+Cd11b+ cells, was observed. This increased liver injury can be explained by increased necroptosis in NLRP3-/- mice, as shown by an increase in mitochondrial ROS and RIPK3. After 28 d however, NLRP3-/- mice have reduced serum transaminases, less inflammation and reduced fibrosis. TUNEL and caspase-3 staining also show decreased cell death in NLRP3-/- mice. Furthermore, caspase-3 staining shows areas of pyroptotic cell death in WT mice after 28 d of BDL. This can be explained due to the inability of NLRP3-/- mice to activate caspase-1 and release IL-1b. Due to the increased RIP3-mediated necroptosis in NLRP3-/- mice expansion of progenitor/oval cells is triggered which could explain the reduced liver injury. In conclusion, in the acute phase NLRP3-/- mice have more liver injury through mitochondrial ROS and RIPK1 – 3 activation, necroptosis and thus increased inflammatory response. Progenitor/oval cell activation mediates tissue repair in chronic phase. In the chronic phase NLRP3-/- mice have less liver injury due to the lack of pyroptotic cell death which leads to less inflammation and fibrosis.

1.31 Tamoxifen is critical as inducer of Cre activity in mouse models for hepatotoxicity studies

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Tamoxifen (TAM) is intensively used for cell-specific Cre recombinase-induced gene (in)activation and reporter gene expression for fate mapping and tracing at predefined time frames. Despite extensive studies using the TAM-cre system, the influence of TAM per se remains to be elucidated. To unravel this influence, male C57Bl/6N mice were subjected to TAM and hepatotoxic (carbon tetrachloride; CCl4) injury. Surprisingly, the level of transaminases (ALT and AST) and necrosis index in TAM/CCl4 mice decreased significantly as compared with the vehicle/CCl4 group. No obvious alterations were reported without damage induction between the groups. mRNA expression of a critical phase I metabolizing enzyme, CYP2E1 was downregulated ($p = 0.0433$) in the TAM pretreated group, as measured by real time RT-PCR. However, protein expression of CYP2E1 as measured by immunostaining and immunoblotting was not influenced by TAM pretreatment. In contrast, anti-oxidants e.g. ascorbate and catalase, as well as some thiol containing metabolites, e.g. methionine, were upregulated in the TAM group upon damage. Furthermore, TAM increases presence of resident macrophages and recruitment of leucocytes in injured areas as indicated by F4/80 and CD 45 positive staining. Our findings suggest that TAM has no clear impact in the control groups; however, upon liver intoxication induces a hepatoprotection via (1) downregulating of CYP2E1 in mRNA level, (2) increasing the availability of antioxidants and thiol group containing metabolites, and (3) recruiting restorative macrophages. This must be considered before conclusions based on this inducible Cre system can be drawn.

1.32 Th2-polarization of CD4+ T-cells is locally induced and drives liver fibrogenesis

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Liver fibrosis arises in the course chronic inflammation following mechanisms that are well-conserved across different etiologies. CD4+ T-cells are key players in the orchestration of immune response. In numerous organs Th2-polarized GATA3+ CD4+ T-cells have been demonstrated to drive fibrosis through the release of profibrogenic cytokines such as IL-13. In the liver Th2-cells can perpetuate matrix deposition in archetypical Th2-diseases, however, it is unclear whether the Th2-fibrosis paradigm can be applied to antigen-independent entities, as well. We aimed at investigating whether Th2 pathways are generally activated in chronic liver inflammation. Whole human liver tissue was obtained from explants during transplantation and tumor resection. Quantitative PCR was performed to study expression of Th2-associated genes. Hepatic CD4+ T-cells were isolated and analyzed accordingly. Using cell lines of different liver resident cells in vitro interaction with CD4+ T-cells under different stimuli was investigated in order to unravel putative mechanisms of local Th2-induction. Furthermore, origin and release of Th2-stimulating cytokines including IL-33 was assessed in vitro and in situ. Various animal models were used to confirm the observations in vivo. Th2-genes were critically regulated in liver fibrosis and cirrhosis largely independent of underlying liver disease. In line, primary hepatic CD4+ T-cells displayed a Th2-like phenotype, paralleled by elevated serum levels of IL-4, IL-13, IL-25, IL-33 and TSLP. Hepatic Il33 and its receptor St2 showed stage-dependent upregulation. Immunofluorescence revealed intimate cross-talk between GATA3+ T-cells and hepatic macrophages and stellate cells. Interestingly, FISH analysis could identify macrophages and biliary progenitor cells as a source of IL-33. Congruently, mouse models with pronounced ductal reaction exhibited highest IL-33 expression. Besides IL-33, we observed reciprocal cell-cell interaction involving hepatocytes under inflammatory conditions leading to a Th2-shift in T-cells and consecutive activation/proliferation of hepatic stellate cells, partially dependent on IL-13. Moreover, primary CD11b+ liver macrophages can also secrete Th2-cytokines upon stimulation. Our data indicate that Th2-polarized CD4+ T-cells are implicated in liver fibrosis. Local inflammatory environment due to soluble factors and cell-cell contact favors a Th2-phenotype mainly through IL33.

1.33 The Epigenetic Modifications by the Histone Demethylase LSD1 in Hepatic Stellate Cells Contribute to Liver Fibrosis

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Background and Aim: Liver fibrosis, representing the final common pathway of all types of chronic liver diseases, is a major public health problem worldwide. After chronic liver injury, Hepatic Stellate Cells (HSCs) differentiate into myofibroblasts, which are central players of liver fibrogenesis. LSD1 is a histone H3 lysine 4 (K4) and lysine 9 (K9) demethylase which acts a key player in carcinogenesis, but its function in liver fibrosis is unknown. Hereby, we aim to study the role of LSD1 and its function in epigenetic modifications during liver fibrogenesis. **Methods:** Therefore, we inhibited LSD1 function in the HSC cell line, HSC-T6, by a reversible LSD1 inhibitor (HCI-2509). In addition, silenced LSD1 expression by a short hairpin (sh) anti-LSD1 RNA using a pSuper.Retro viral transduction system, generate stable polyclonal and monoclonal LSD1 knockdown HSC cell lines. Expression profilingg of LSD1 silenced HSC was performed by microarrays. Gene and miRNA expression was further quantified by qPCR and changing in the histone modification was studied by Western blot. **Results:** In both, HSC knockdown cells and in HSC treated with the HCI-2509 LSD1 inhibitor, LSD1 downregulation resulted in a decreased of epigenetic writers and erasers HDAC3, EZH2, and Mecp2, shown previously to be involved in fibrotic features. In addition we demonstrate that LSD1 silencing causes an altered gene and miRNA expression profile in HSC. Thus, the fibrogenic markers collagen I and SMA were decreased, whereas PPARγ was enhanced. LSD1 mediated alteration of fibrotic components was associated with an altered miRNA pattern, in particular with an increase of the anti-fibrotic miRNA29a, which targets collagen biosynthesis. **Conclusions:** In our studies, we collected evidence that LSD1 plays a central role in HSCs, contributing to liver fibrosis. Anti-fibrotic function of LSD1 is suggested to be mediated by its influence on miRNA expression and its impact on other epigenetic modifiers.

1.34 The Role of IFNγ in the Immune Pathogenesis of Primary Sclerosing Cholangitis

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Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease, characterized by proliferation of the bile ducts that is accompanied by inflammatory bowel disease in two thirds of the patients. High levels of Interferon-γ (IFNγ) and IFNγ-induced chemokines have been detected in liver tissue and plasma of PSC patients. Aim of this study is to analyze the role of IFNγ-producing or IFNγ-activated cells in the immune pathogenesis of PSC. Therefore, IFNγ was neutralized in multidrug resistance protein 2 knockout (Mdr2-/-) mice representing a model that resembles PSC. Mdr2-/- mice (10 weeks old) were treated with anti-IFNγ antibody for 2 weeks. Histological analysis allowed the examination of inflammation and fibrosis. To investigate the cytotoxic potential of non-parenchymal liver cells (NPCs) after restimulation with PMA/Ionomycin, in vitro studies were performed and analyzed by FACS. Real time PCR was applied to examine the gene expression of targets involved in the IFNγ signaling pathway. The fibrosis level of anti-IFNγ treated mice was reduced compared to the IgG control treated mice. Restimulation of NPCs showed a reduced frequency of IFNγ-producing NK cells and CD8+ T cells. Both cell populations expressed less CD107 indicating reduced cytotoxicity. In addition, the anti-IFNγ treated mice showed reduced hepatic mRNA expression levels of transcription factors like STAT1, SMAD7 and IRF1. In conclusion, neutralization of IFNγ in Mdr2-/- mice showed a reduction in liver fibrosis and lymphocytes cytotoxicity. This allows the assumption, that IFNγ is involved in the immune pathogenesis of PSC. Further studies will reveal the specific cell populations involved.

1.35 Thyroid Hormone Receptor (TR): a regulator in Liver Fibrogenesis

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Thyroid hormone (TH) signaling is critical for tissue-organ development, growth, differentiation and metabolism, and in the liver, the most widely expressed TH receptor is TRβ. In a recent study of patients with nonalcoholic steatohepatitis (NASH), progressive liver fibrosis was associated with disrupted TH signaling. In a separate study, low serum triiodothyronine (T3) was associated with advanced NASH-fibrosis. These findings suggest that TH signaling may be a novel regulator of adult liver fibrogenesis. Herein, we hypothesized that the TH-TR axis modulates HSC phenotype and perturbations in TH-TR axis occur during liver injury. **Methods:** In vivo: Two murine liver injury models were used (6 weeks of methionine choline deficient (MCD) diet and 6 weeks carbon tetrachloride (CCL4) injection). Human tissues were obtained from explanted NASH and healthy donor livers. Liver fibrosis was assessed by Sirius Red (SR) staining, αSMA immunohistochemistry, and the hepatic hydroxyproline assay. Total liver expression of TRα and TRβ, αSMA and Collagen 1α1 mRNA were determined by qRT-PCR. In vitro: To determine whether TH modulates HSC activation, primary HSC and a HSC line were treated with transforming growth factor (TGF)-β (5 ng/ml), in the presence or absence of T3 (10 ng/ml). TRα knockdown in HSC was achieved using

lentiviral-mediated shRNA (shTRα). Results were confirmed using TRα knock-out (TRαKO) mouse embryonic fibroblasts (MEF). HSC migration was assessed using the wound healing assay, western blot and qRT-PCR (αSMA, collagen 1α1, TRα and β). Hepatocytes were used as positive control for TRβ expression. **Results:** Total liver TRα and TRβ mRNA were downregulated by 4 fold ($p < 0.05$) during liver fibrogenesis in mice and humans. In HSC, TRα is the predominant TR (4 fold higher than TRβ; $p < 0.05$). TGF-β stimulation of HSC repressed TRα and TRβ mRNA expression, but this effect was blunted by T3. shTRα-HSC exhibited greater activation at baseline, with increased fibrogenesis markers αSMA and collagen 1α1, and enhanced responses to TGF-β (increased p-Smad2/3). shTRα-HSC also had greater response in wound-healing assay (1.3 fold, $p < 0.05$), which was consistent with enriched contractility/migration pathways obtained from RNA sequencing analysis. **Conclusions:** Liver expression of TRα and β is repressed during fibrogenesis following chronic liver injury. TRα is the predominant TR in HSC and perturbations in TH-TR levels regulate HSC phenotype via the TGF-β pathway. Future studies will be needed to determine if whether TH treatment could inhibit liver fibrosis progression.

1.36 Time course Analyse der miRNA-Expression bei der PDGF-B vermittelten Leberfibrogenese

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Hepatozytenspezifische Überexpression von PDGF-B induziert eine Leberfibrose. Um die zugrunde liegenden genetischen Mechanismen zu analysieren wurden männliche PDGF-B überexprimierende und entsprechende Wildtypkontrolltiere zu verschiedenen Altern (4, 12, 24 und 32 Wochen) mittels miRNA-Sequenzierung auf einem Illumina HiSeq1000 und anschließender MirDeep2- und DESeq2-Analyse untersucht. Auf diese Weise konnten multiple im Zeitverlauf differentiell regulierte miRNAs identifiziert werden, die eine konstant signifikant erhöhte Expression (z. B. miRNA-34-, 125, -181, -199, -let7b) oder auch verminderte Expression (z. B. miRNA-101, -194) aufwiesen. Diverse miRNAs weisen im Zeitverlauf jedoch auch zum Teil erhebliche Änderungen ihrer Expression auf. So verringert sich z. B. für miRNA-92, -322, oder -let7i die Expression im Zeitverlauf oder weisen z. B. miRNA-20, -26, -27 im Verlauf ansteigende Tendenzen auf. Einige zuvor als PDGF-B regulierend beschriebene miRNAs wie miRNA-19, -29 und -148 fanden sich in unserem Modell nicht reguliert. Dies mag auf einen sehr frühen Mechanismus der Fibrogenese oder negative feed back Mechanismen hindeuten. Hingegen zeigt sich die Expression einer Reihe von miRNAs, welche durch PDGF-B regulierten differentiell verändert werden, so z. B. miRNA-106, -130 und -34 in ihrer Expression hochreguliert. Schließlich fand sich auch für diverse mit der Hepatokarzinogenese in Kontext stehende miRNAs, wie miRNA-23, -106, -193 und -223, ein differentiell reguliertes Muster. Letzteres deutet auf eine frühe, spontane Induktion der Karzinogenese in diesen Tieren hin. Zusammenfassend zeigt sich, dass die der PDGF-B vermittelten Leberfibrogenese zugrundliegenden molekularen Mechanismen auch auf einer posttranskriptionellen Regulation durch Veränderungen bei der miRNA-Expression beruhen und im Zeitverlauf eine differenzierte Regulation erfahren.

2. Clinical Hepatology

2.1 Interpretation of follow-up 3D-MRCP/MRI in patients with primary sclerosing cholangitis

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Background: In primary sclerosing cholangitis (PSC) many centers perform regular follow-up magnetic resonance cholangiopancreatography (MRCP) and magnetic resonance imaging (MRI), particularly to early detect bile duct pathologies. To date, no standardized MRI protocols for PSC have, however, been established, nor do MRI-based definitions (e.g. of dominant strictures) exist. Thus, the interpretation of follow-up MRCP/MRI and MRI-based recommendations for/against endoscopic retrograde cholangiopancreatography (ERCP) may significantly vary between physicians. We therefore aimed to evaluate the interpretation of follow-up MRCP/MRI for PSC among PSC experts. **Methods:** Members/associates of the international PSC study group were invited to an online-survey (surveymonkey.com) consisting of 16 real-life PSC cases. Each case included essential clinical and biochemical information and video material of follow-up 3D-MRCP/MRI. Using a multiple-choice questionnaire, participants were asked to interpret 3D-MRCP/MRI and for recommendations, particularly with respect to ERCP. The agreement among the participants was calculated using Fleiss-kappa. **Results:** 44 participants (19 hepatologists, 16 gastroenterologists, 9 radiologists) with a median PSC experience of 14 years completed the survey. With respect to the overall assessment, presence of dominant strictures and suspicion of cholangiocarcinoma the agreement reliability analysis revealed only a slight agreement among the group. The lowest agreement was found with respect to ERCP recommendation (yes/no; Fleiss-kappa = 0.12, 95% CI 0.11 – 0.14), with a relative agreement > 75% in only 4/16 cases. **Conclusion:** Our study demonstrates that in PSC the interpretation of follow-up 3D-MRCP/MRI and MRI-based recommendations significantly vary even among experienced practitioners. Standardized MRI protocols/definitions and standards for ERCP indication are required for the management of PSC patients.

2.2 Multizentrische Studie zur Leberbeteiligung beim Alpha1-Antitrypsin-Mangel

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Hintergrund: Der Alpha1-Antitrypsin(AAT)-Mangel wird durch eine AAT-Mutation verursacht und prädisponiert zum Lungenemphysem sowie zur Entwicklung einer Leberzirrhose. Die Leberbeteiligung bei AAT-Mangel wird häufig übersehen und es gibt keine Standards zur Frühdiagnostik und Vorsorge. Um dies zu ändern und das Ausmaß der Lebererkrankung systematisch zu erfassen, haben wir eine multizentrische, internationale Studie initiiert. **Patienten & Methodik:** In Kooperation mit Patientenvereinigungen und spezialisierten Pneumologen wurden 141 Patienten mit einem schweren AAT-Mangel (homozygoter PiZZ-Genotyp) sowie 50 gesunde Kontrollen ohne AAT-Mutation aus 4 Ländern eingeschlossen. Die Studienteilnehmer werden prospektiv hinsichtlich epidemiologischer, anamnestischer, laborchemischer und elastographischer Parameter untersucht. Die Lebersteifigkeit sowie Controlled Attenuation Parameter (CAP) wurden mit transienter Elastografie (TE) gemessen. **Ergebnisse:** 82,3% der Patienten gaben an, keine regelmäßigen Leberuntersuchungen zu erhalten, obwohl sie regelmäßig pneumologisch betreut werden. Bei 20% (14% Frauen, 26% Männer) der PiZZ-Patienten lag die ALT, bei 12% (12% Frauen und Männer) war die AST erhört. Die Lebersteifigkeit der PiZZ-Patienten lag mit 6,8 ± 3,9 kPa höher als bei Kontrollen (4,7 ± 1,0 kPa, $p < 0{,}0001$). PiZZ-Männer hatten eine höhere Lebersteifigkeit als PiZZ-Frauen (7,9 ± 4,8 kPa vs. 5,7 ± 2,1 kPa, $p < 0{,}001$). ALT- und AST-Werte korrelierten nur schwach mit der Lebersteifigkeit (jeweils $r = 0{,}21$). Die CAP als Surrogat der Steatose war bei PiZZ-Patienten erhöht (273 ± 69 vs. 236 ± 51 dB/m, $p < 0{,}01$). Die CAP korrelierte mit dem BMI ($r = 0{,}60$, $p < 0{,}0001$), allerdings korrelierten weder CAP noch BMI signifikant mit der Lebersteifigkeit. Die Lebersteifigkeit korrelierte nicht mit pulmonaler Symptomatik (COPD Assessment Test). Zudem zeigte sich kein signifikanter Unterschied der Lebersteifigkeit zwischen Patienten mit und ohne Heimsauerstofftherapie bzw. mit und ohne intravenöser AAT-Substitution. **Schlussfolgerung:** PIZZ-Patienten (insbesondere PiZZ-Männer) neigen zur Entwicklung einer Leberfibrose, die mit Routine-Untersuchungen nicht erfasst wird. Dies scheint entgegen weitläufiger Meinung unabhängig von der Lungenfunktion zu sein. Erhöhte CAP-Werte bei PiZZ-Patienten weisen auf eine vermehrte Leberverfettung hin, diese Daten sollten aber noch mit unabhängigen Methoden validiert werden.

2.3 Therapeutic Vaccination with a Third Generation PreS/S vaccine (Sci-B-VacTM) with HBV Carriers with Low-level of HBsAg result in Serovonversion to Anti-HBs

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Background & Aims: Chronic hepatitis B (CHB) is currently treated by IFN-α and nucleos(t)ide analogues (NUCs), however, without satisfactory results. The major problem consists in the persistence of a cccDNA in patients. Several new antiviral drugs have been developed in the recent years; however, they rarely reduce the copies of cccDNA. Therefore, cytolytic and noncytolytic approaches are needed to eliminate cccDNA from HBV-infected hepatocytes. Effective virusspecific T and B cell immune responses remain crucial in eliminating cccDNA-carrying hepatocytes and the long-term control of HBV infection. Reduction of HBV viremia by antiviral drugs provides a window for reconstitution of HBV specific immune response. Based on data obtained in our preclinical studies the combination of antiviral drugs and immunization with a third generation PreS/S vaccine (Sci-B-VacTM) may control HBV viremia during drug-off period of patients. **Methods:** We immunized 4 HBsAg positve Patients with chronic Hepatitis B with Sci-B- VacTM 3-to 6 times and determined HBsAg (IU/L) and the anti-HBs antibody titers (IU/L) before immunization and after each vaccination. All patients were treated with nucleos(t)ide analogues (NUCs) at least for two years and were HBV DNA negative prior to vaccination. **Results:** HBsAg concentrations before the first vaccination of the 4 immunized were 19812, 448, 20.2, 19.2 IU/l, respectively. All patients remained HBV DNA negative for the whole period of observation (at least 2 years). After three vaccinations 3 of the 4 patients seroconverted to anti-HBs. One year after onset of vaccination that antibody concentrations were 140, 80 and 150 IU/L, respectively. The patient with an initial high concentration of HBsAg did not seroconvert. CD4 and CD8 T cell response is currently under investigation and will be presented at the meeting. **Conclusion:** The HBV carriers with low-level of HBsAg and negative results for HBV DNA under NUC treatments receiving therapeutic vaccination with immunization with a third generation PreS/S vaccine (Sci-B-VacTM) seroconverted to anti-HBs. This may therefore reduce the tolerizing effect of HBsAg with respect to an appropriate T cell response. Further studies in a larger cohort including long term observation are needed to determine whether HBV cccDNA of these patients may be reduced or even eliminated.

2.4 Shear wave elastography of the liver and spleen in patients with autoimmune hepatitis and its variants – A single centre study

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Background: Autoimmune hepatitis (AIH) is a chronic liver disease which might lead to advanced fibrosis and cirrhosis. Unlike in viral etiology, data on elastographic assessment of liver fibrosis in AIH is scant and virtually do not exists in terms of spleen elastography. The aim of this study is to evaluate the potential association between shear wave elastography (SWE) of the liver and spleen with various parameters including serum fibrosis markers in patients with autoimmune hepatitis (AIH) and AIH variants. **Materials and Methods:** Fifty consecutive in and out-patients (M/F (16/34, mean age: 37 yrs.) with AIH and its PSC and PBC variants defined according to recent EASL Clinical Practice Guidelines (J Hepatol 2015) were included. They underwent liver and spleen SWE (SuperSonic Imagine Aixplorer®). Different biochemical tests were performed including fibrosis markers and non-invasive tests: Fibrosis-4, aspartate aminotransferase (AST)-to-platelet ratio index (APRI), AST-to-alanine aminotransferase (ALT) ratio (AAR), APRI, FibroQ and Model For End-Stage Liver Disease (MELD) score. Pearson correlation coefficients analysis with was performed and p values < 0.05 were considered significant. **Results:** In total, 13 patients (26%) fulfilled elastographic criteria for diagnosing liver cirrhosis. Liver SWE showed a significant correlation with spleen SWE ($p < 0.05$), AST ($p<.001$), ALT ($p<.01$), alkaline phosphatase (ALP) ($p < 0.001$), INR ($p < 0.001$) and MELD ($p < 0.001$), Fibrosis-4 ($p < 0.001$), APRI ($p < 0.001$) and Fibro-Q ($p < 0.05$). There was also a significant, negative correlation between Liver SWE and platelet count ($p < 0.01$) and serum albumin ($p < 0.001$). Notably, spleen SWE correlated with Fibrosis-4 ($p < 0.05$) and showed a negative correlation with platelet and serum albumin ($p < 0.05$). **Conclusions:** Liver SWE could be of use in a non-invasive assessment of liver fibrosis in patients with AIH and its variants. The possible role of spleen SWE in the assessment of portal hypertension in AIH has to be established in larger cohorts of patients, however, present results might suggest that it can potentially serve as an additional modality in evaluation of patients suffering from liver autoimmune diseases.

2.5 NOD2 genetic variants confer risk for secondary sclerosing cholangitis in critically ill patients

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Introduction: Sclerosing cholangitis in critically ill patients (SC-CIP) is a progressive cholestatic disease. Its etiology is unknown. It has been hypothesized that biliary infections might be involved in the pathogenesis. Hence in the current study we investigate if common NOD2 (nucleotide-binding oligomerisation domain containing 2) gene variants, which we previously identified to represent not only risk factors for Crohn's disease but also for bacterial translocation in liver cirrhosis, increase the odds of developing SC-CIP. **Patients and methods:** We screened a total of 4,641 endoscopic retrograde cholangiography procedures and identify 17 patients (14 men, median age 63 years) with SC-CIP (Cohort 1, discovery cohort), who were then genotyped for the three common NOD2 mutations. To validate the association, we subsequently tested these NOD2 variants in 29 patients from SC-CIP cohorts of three additional academic medical centers (Cohort 2, replication cohort). In addition, we genotyped specific polymorphisms of hepatocanalicular transporter genes to investigate their role in the development of SC-CIP. **Results:** In Cohort 1, the NOD2 variants were present in 5 of 17 SC-CIP patients (29.4%), which is twice the frequency of the general population. Four patients carried the p.R702W variant and one patient harbored the c.3020insC variant. These results were replicated in Cohort 2 with 8 SC-CIP patients (27.6%) showing NOD2 mutations. In contrast, polymorphisms in hepatocanalicular transporter genes did not have major impact on SC-CIP risk. **Conclusion:** This first study on genetic susceptibility in SC-CIP patients shows an extraordinary high frequency of NOD2 variation. These results suggest a pivotal role of inherited impaired anti-bacterial defense in the development of this devastating biliary disease.

2.6 3 Jahre Real-Life Essener Erfahrungen einer Everolimus-basierten Immunsuppression nach Lebertransplantation

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Hintergrund: Seit der Zulassung neuer immunsuppressiver Therapien nach Lebertransplantation beim Erwachsenen werden zunehmend Patienten auf CNI-reduzierte oder CNI-freie Regime umgestellt. Der mTor-

Inhibitor Everolimus ist zur Immunsuppression nach Lebertransplantation für Erwachsene seit 2012 zugelassen. Wir haben die Verträglichkeit sowie den Verlauf verschiedener klinischer Parameter nach Umstellung auf ein EVR-basiertes Regime dokumentiert und retrospektiv ausgewertet. **Material und Methoden:** Es wurden 263 Patienten von einer CNI-Monotherapie auf ein CNI reduziertes oder CNI-freies Regime umgestellt. Es wurden Parameter zur Lebensqualität und Adhärenz anhand des Essener Fragebogens erhoben. Parallel wurden klinische Parameter und Beschwerden der Patienten dokumentiert. Sämtliche Parameter wurden über einen Zeitraum eines Jahres mehrfach erhoben und in eine Datenbank eingegeben, um Veränderungen der Parameter im Zeitverlauf nach Umstellung der Immunsuppression festzuhalten. **Ergebnisse:** Insgesamt konnten von 151 Patienten die Daten 12 Monate nach Umstellung und von 112 Patienten die Daten 6 Monate nach Umstellung ausgewertet werden. Insgesamt wurden 113 Patienten (43%) im Zeitraum 6 – 24 Monate nach LTx umgestellt. Zudem wurden 94 Patienten (36%) > 24 Monate nach LTx und 56 Patienten (21%) bereits während der ersten 6 Monate nach LT umgestellt. Die Therapieadhärenz hat sich leicht verbessert, ohne statistische Signifikanz zu erreichen. Wobei die gleichzeitig abgefragte Lebensqualität nach Umstellung der Immunsuppression deutlich zugenommen hat. Nur 30 Patienten mussten die Medikation aufgrund von Nebenwirkungen abbrechen. Dabei wurde die Therapie anteilig schlechter vertragen in der Gruppe welche, innerhalb der ersten 6 Monate umgestellt wurde. Bezogen auf die Indikation zur LT, wurde bei Patienten mit einer viral bedingten Zirrhose als Grunderkrankung häufiger eine eher schlechtere Verträglichkeit und Lebensqualität festgestellt. Die klinischen Parameter, insbesondere die Nierenfunktion und die metabolischen Parameter haben sich nach Umstellung der Immunsuppression stabilisiert bis verbessert. Die Leberparameter sind konstant geblieben und es hat sich kein Ereignis einer akuten Abstoßung ereignet. **Schlussfolgerung:** Die Umstellung auf ein CNI-reduziertes oder CNI-freies immunsuppressives Regimen nach Lebertransplantation, unter Einsparung von Tacrolimus, bedeutet in den meisten Fällen eine deutliche Verbesserung der Lebensqualität wie auch der klinischen Parameter und kann ohne Einschränkung oder Gefährdung der Transplantatfunktion durchgeführt werden.

2.7 Evaluation eines Faktor XIII-Mangels mittels Rotationsthrombelastometrie (ROTEM®) bei Leberzirrhosepatienten mit Blutungsneigung

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Einleitung: Blutungsereignisse sind gefürchtete Komplikationen bei Patienten mit Leberzirrhose. Ob bzw. in welchem Ausmaß konventionelle Gerinnungstests (INR, aPTT, Fibrinogen) in diesem besonderen Patientenkollektiv eine Blutungsneigung erfassen können, wird kontrovers diskutiert. Die Rotationsthrombelastometrie (ROTEM®) stellt heutzutage eine innovative Evaluationsmethode der Gerinnungsfunktion dar. Ob sie einen Faktor XIII-Mangel akkurat abbilden kann, wurde bei Patienten mit Leberzirrhose noch nicht ausreichend evaluiert. **Methodik:** In dieser Studie wurden die Daten von 74 Patienten mit Leberzirrhose retrospektiv erfasst und ausgewertet. Diese umfassten die ROTEM®-Analyse, Faktor XIII, konventionellen Gerinnungsparameter, klinische Scores (CHILD, MELD) und Blutungsereignisse. **Ergebnisse:** 43 Patienten (58,11%) zeigten einen Faktor XIII-Mangel (< 70%). Dieser Mangel korrelierte mit der Gerinnselfestigkeit der ROTEM®-Analyse, wie MCFextem and MCFfibtem ($r = 0{,}48$, $p < 0{,}0001$ und $r = 0{,}60$, $p < 0{,}0001$). Die Maximale Lyse zeigte keine Korrelation zu dem Faktor XIII-Mangel ($r = -0{,}14$, $p = 0{,}29$ für MLextem und $r = -0{,}02$, $p = 0{,}88$ für MLaptem). Patienten mit hämorrhagischer Diathese (n = 22, 29,73%) zeigten signifikant niedrigere FXIII-Spiegel ($p < 0{,}0001$), sowie Gerinnselfestigkeit in der ROTEM®-Analyse ($p = 0{,}0055$ für MCFextem und $p = 0{,}0013$ für MCFfibtem). Die Gerinnungszeit in EXTEM war bei Patienten mit Blutungsneigung signifikant verlängert ($p = 0{,}0403$). **Schlussfolgerung:** Der Faktor XIII-Mangel tritt häufig bei Patienten mit Leberzirrhose auf. Durch die ROTEM®-Analyse kann indirekt dieser Mangel abgebildet werden. Ein FXIII-Mangel, die verlängerte Gerinnungszeit in EXTEM, sowie die reduzierte Gerinnselfestigkeit der ROTEM®-Analyse sind mit Blutungskomplikationen assoziiert.

2.8 A non-invasive comparison between NASH and Crohn's disease

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Hepatic steatosis and non-alcoholic steatohepatitis (NASH) show an increasing prevalence in multiple alimentary tract diseases, including inflammatory bowel diseases (IBD) as indicated in recent studies while we and others have shown that NASH in IBD patients is associated with an increased susceptibility for acute-on-chronic liver failure. So far, little is known about the underlying mechanisms inducing hepatic steatosis in IBD. We aimed to analyze and compare non-invasive markers of liver injury in patients with NASH and individuals with Crohn's disease (CD). Here, we included patients with established NASH and those with established CD without a history of liver disease and analyzed serum parameters of liver injury and hepatocellular apoptosis (M30), a breath test for small intestinal bacterial overgrowth (SIBO) and transient elastography as well as controlled attenuation parameter (CAP) to characterize hepatic steatosis. Patients with NASH had a significantly higher BMI compared to CD, expectedly. Hence, ALT and AST levels as well as M30 were significantly higher in NASH vs. CD. SIBO occurred in only few individuals and did not affect steatosis. Interestingly, while transient elastography revealed increased liver stiffness in NASH vs. CD, there was no significant difference in CAP as an assessment for hepatic steatosis comparing the groups. Actually, 43.8% of CD patients had a CAP > 283dB/m, defined as a previously established cutoff value for significant hepatic steatosis with a maximum CAP of 400dB/m in one patient. In general, transaminases were within normal limits in most patients but in CD patients with CAP > 283dB/m AST and ALT levels were significantly higher compared to CD patients with lower CAP results. Thereupon we reviewed the patients' drug regimens in order to determine potential influences resulting in higher CAP values. Much to our surprise we found steroid therapy was not associated with CAP in this cohort. However, individuals with CAP below 283dB/m were more likely to be treated with biologicals. Here we revealed that CAP was significantly lower in patients treated with biologicals as compared to other treatment plans (237.3 ± 11.7 vs. 306.2 ± 20.6 dB/m; $p < 0.05$). Thus, in this cohort, NASH was associated with higher BMI, transaminase as well as M30 levels and liver stiffness, while hepatic steatosis as assessed by CAP was not pronounced compared to CD. In CD patients with significant steatosis, higher transaminase levels indicate subliminal hepatic inflammation, despite being within normal levels. Treatment with biologicals seems to protect CD patients from hepatic steatosis. In conclusion, we identified a high rate of hepatic steatosis in CD with alterations in transaminase levels and a potential association with biologicals.

2.9 A TLR9 promoter polymorphism modulates the monocytic immune responses in decompensated cirrhosis

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Background: The presence of circulating and peritoneal fragments of bacterial DNA alongside increased pro-inflammatory and anti-inflammatory cytokines is a hallmark finding in advanced cirrhosis and acute-on-chronic liver failure (ACLF). Polymorphisms in the promoter region of toll-like receptor 9 (TLR9), an endosome receptor recognizing unmethylated CpG-rich bacterial DNA, may contribute to hyper-inflammatory immune responses in advanced cirrhosis. **Aims:** To investigate the consequences of the single nucleotide polymorphism rs5743836 in the promoter region of TLR9 on inflammatory responses in monocytes and macrophages. **Methods:** Whole blood from patients with decompensated cirrhosis was genotyped by endpoint Taqman-PCR. Monocyte and macrophages were isolated from patient blood and ascites using density gradient centrifugation followed by immunomagnetic sorting. Lipopolysaccharides (LPS) and CpG-rich single stranded DNA oligonucleotides

(CpG-ODN) were used for in vitro stimulation of CD 14-positive cells. mRNA expression was quantified using relative RT-qPCR. **Results:** The minor allele frequency of the polymorphism in patients with decompensated cirrhosis was comparable to the general population (0.14). TLR9 expression in monocytes from patients carrying the TLR9 promoter variant tended to be lower than in wild-type patients ($p = 0.09$) but significantly increased after In vitro stimulation with LPS ($p = 0.027$). CpG-ODN-induced IL 6 ($p = 0.003$) and IL 10 ($p = 0.030$) mRNA expression in monocytes and IL 10 mRNA expression in macrophages ($p = 0.05$) was increased in patients with the TLR9 promoter polymorphisms as compared to the wild type. **Conclusions:** Patients with cirrhosis carrying the frequent TLR9 promoter variant show hyper-inflammatory responses after stimulation with CpG-rich bacterial DNA. Studies are ongoing to investigate whether this association translates into increased complications in clinical practice.

2.10 Accurate standardization and normalization of hepatic metal contents in Laser Ablation Inductively Coupled Plasma Mass Spectrometry (LA-ICP-MS)

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Background: LA-ICP-MS is a powerful methodology for the precise determination and quantification of metals in nearly any kind of sample [1]. We recently used this method to quantify metals in experimental models and clinical samples of Wilson's disease [2 – 3]. However, measured ion intensities of selected mass-to-charge (m/z) ratios, may vary considerably from measurement to measurement and underlie non-linear drift during time. Beside proper calibration, normalization strategies for measured traces of a specific ion to a well-characterized reference material are required. Other strategies use an endogenous reference element that ideally should have a homogenous distribution within the measured sample. However, normalization methods depend on the chosen experimental setup, the sample material analyzed, and are most often based on one or few isotopes or the total ion current. **Methods:** We here compared different normalization methods that either used a separate reference value for each data point – constituting a pixel in the isotope image – or used a constant normalization factor per measurement run. For image generation and visualization of element concentrations, we used Microsoft Excel 2010 with the novel software tool "Excel Laser Ablation Imaging" that is based on Microsoft Excel Visual Basic for Applications [4]. Matrix-matched standards were produced from homogenized tissue that is homologous to the sample and spiked with different concentrations of standard solutions served for calibration. **Results:** Best results were obtained using individual isotopes or isotope groups as reference. The total ion current was not suitable for inorganic mass spectrometry as Na and K which dominate the spectrum. In comparison to normalization with an overall factor, pixel per pixel normalization may increase the image noise, but it can be used to attenuate measurement errors and signal drift of long-lasting measurements. Isotopes with low signal-to-noise ratios may show an increased background noise after normalization, which can be significantly reduced by defining an area of interest. With these standardization and normalization we now are able to precisely quantify hepatic metal alterations that are observed in metal overload disease (e.g., Wilson's disease, hemochromatosis, zinc overdose). **Conclusions:** The normalization in LA-ICP-MS measurements is essential to minimize deviations of element concentrations that might occur as the consequence of measurement-related fluctuations. The data sets we present show that proper normalization and the definition of an area of interest that is taken for quantification are powerful tools to obtain high-contrast isotope images with precise metal concentrations. These methods are helpful to quantify metal overload in hepatic diseases. **References:** [1] Susnea I, Weiskirchen R. Mass Spectrom Rev. 2015 Feb 11. doi: 10.1002/mas.21454 [Epub ahead of print]. [2] Boaru SG, Merle U, Uerlings R, Zimmermann A, Weiskirchen S, Matusch A, Stremmel W, Weiskirchen R. BMC Neurosci. 2014;15:98. [3] Boaru SG, Merle U, Uerlings R, Zimmermann A, Flechtenmacher C, Willheim C, Eder E, Ferenci P, Stremmel W, Weiskirchen R. J Cell Mol Med. 2015;19:806 – 14. [4] Uerlings R, Matusch A, Weiskirchen R. Int J Mass Spectrom. 2016;395:27 – 35.

2.11 Albumin creatinine ratio predicts medical intensive care unit outcome of patients with hepatic encephalopathy

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Background and Aims: Hepatic encephalopathy (HE) in a hospitalized cirrhotic patient is associated with a high mortality rate, unless treated; the 1 st year survival is 42%. Increasing evidence has indicated that in patients with cirrhosis, proteinuria is associated with reduced survival compared with patients without proteinuria. We attempted to explore the prognostic role of proteinuria in patients with hepatic encephalopathy admitted to medical intensive care unit (MICU). **Methods:** 78 consecutive cirrhotic patients with HE admitted to MICU included. Patient's demographics, HE grade, ChildPugh class, SOFA score, MELD score and albumin/creatinine ratio (ACR) were documented at the 1 st day of MICU admission for all patients. MICU mortality, the occurrence of acute kidney injury (AKI) and respiratory failure during MICU admission also recorded. **Results:** Among 78 cirrhotic patients with HE (39 females, and 39 males), There mean age was 57.65 ± 12.9 years. HE grades 0, I, II, III, and IV detected among 0, 0, 16 (20.5%), 44 (56.4%) and 18 (23%) patients respectively. 0, 12 (15.4%) and 66 (84.6%) patients had Child classes A, B and C respectively. The mean MELD and SOFA scores were 17.97 ± 6.36 and 6.89 ± 2.71respectively. In addition the mean ACR among included patients was 109.54 ± 138.48 µg/dl. The mean MICU stay of all patients was 4.78 ± 2.81 days. MICU mortality rate was 32% (n = 25). 23 (29.5%), and 7 (9%) patients developed AKI and respiratory failure, during MICU stay, respectively. ACR > 57 µg/dl was the best cut-off value for prediction of MICU mortality. The accuracy, sensitivity, specificity, positive predictive value, and negative predictive value of ACR > 57 µg/dl, for prediction of MICU mortality was 83%, 76%, and 75.5%, 59.4% and 87% respectively. Interestingly ACR perfectly discloses patients with pre-renal AKI from those with of AKI due to renal causes. **Conclusions:** ACR is a promising Predictor of prognosis in patients with HE. In addition it can be used as early marker of AKI due intrinsic kidney disease in HE patients admitted to MICU. Keyword: Prognosis of hepatic encephalopathy in MICU

2.12 Association of controlled attenuation parameter (CAP) and HbA1c in patients with fatty liver

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Background and aim: Chronic liver diseases and serum glycosylated hemoglobin (HbA1c) levels are linked to one another through the metabolic syndrome. Our aim now was to analyze for the first time the potential association between hepatic steatosis, as determined quantitatively with the controlled attenuation parameter (CAP), and HbA1c. **Patients and methods:** At a tertiary referral center in Germany, we evaluated a group of 212 outpatients with hepatic steatosis retrospectively, of whom 93.4% presented with non-alcoholic liver disease. Hepatic steatosis was assessed non-invasively using controlled attenuation parameter (CAP), which quantifies the degree of ultrasound attenuation based on vibration-controlled transient elastography (Fibroscan). Serum HbA1c and liver function tests were measured with standardized clinical chemistry assays. The NAFLD susceptibility variant PNPLA3 p.I148 M was genotyped using Taqman assays. **Results:** Overall in this cohort (113 men, median age 52 years), median CAP was 293 dB/m (100 – 400), and 171 (80.7%) patients presented with elevated CAp ≥ 238 dB/m, indicating marked hepatic steatosis. Median BMI was 30.2 kg/m2 (17.2 – 47.4), median HbA1c was 5.6% (3.7 – 10.4), and serum ALT activities were 45 U/l (9 – 301). The frequency of elevated CAP increased with higher serum HbA1c levels ($rs = 0.230$, $P = 0.001$). Patients with both hepatic steatosis and increased HbA1c levels (HbA1c ≥ 6.0%) displayed significantly ($P = 0.001$) higher CAP values as compared to those with normal levels (312 vs. 286 dB/m). As compared to non-diabetics, CAP values and HbA1c levels were higher in diabetics (322 vs. 282 dB/m and 6.8 vs. 5.3%, both $P < 0.001$). In our cohort, 104 patients (49.0%) carried at least one PNPLA3 p. 148 M risk allele. When stratifying for PNPLA3 genotype, the genetic association was maintained for carriers of the risk allele p. 148 M and normal levels of HbA1c ($P < 0.001$) but not for those with increased levels. Overall, the risk for hepatic steatosis was independently associated

with HbA1c, BMI, ALT and age as determined by multivariate linear regression analysis (all $p \leq 0.013$). **Conclusions:** Non-invasive risk stratification and follow-up of fatty liver in patients with metabolic syndrome is needed because of potential progression to steatohepatitis. Steatosis as assessed by CAP is associated with HbA1c in non-diabetic individuals, and the combination of these non-invasive markers improves individual risk assessment of patients with chronic liver diseases.

2.13 Carcinogenic Etheno DNA-Adducts in Alcoholic Liver Disease: Correlation with Cytochrome P-450 and Fibrosis

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Background & Aims: One mechanism by which alcoholic liver disease (ALD) progresses from pure fatty liver to fibrosis and cirrhosis is oxidative stress and the generation of reactive oxygen species (ROS), predominantly due to the induction of cytochrome P-4502E1 (CYP2E1). Experimental data underline the key role of CYP2E1 since ALD could be partially prevented in rats by the administration of the specific CYP2E1 inhibitor chlormethiazole. Since CYP2E1 is responsible for the formation of carcinogenic etheno DNA-adducts in man and since CYP2E1 inhibition in rats results in a significant reduction of hepatic adenomas, a causal role of alcohol induced CYP2E1 in hepatocarcinogenesis has been recognized. The purpose of this study was to investigate CYP2E1 induction in ALD and to correlate CYP2E1 with various DNA lesions and histological parameters including hepatic fat, inflammation, fibrosis. **Methods:** Hepatic biopsies from 97 patients with a long standing alcohol history (> 60 g/day over 20 years) diagnosed as ALD were histologically scored for steatosis, inflammation and fibrosis. In addition, CYP2E1, the exocyclic etheno-DNA adduct 1,N6-etheno-2'deoxyadenosine (εdA), and 8-hydroxydesoxyguanidine (8-OHdG) were determined immunohistochemically and correlated to each other. **Results:** A significant correlation was found between CYP2E1 and εdA ($p < 0.0001$) as well as CYP2E1 and 8-OHdG ($p = 0.039$). Both CYP2E1 ($p = 0.0094$) and εdA ($p < 0.0001$) also correlated significantly with the stage of hepatic fibrosis. Furthermore, a significant correlation between the stage of fibrosis and the grade of lobular inflammation ($p < 0.0001$), as well as body mass index ($p = 0.006$) was observed. However, the amount of alcohol consumed did not correlate with any of the parameters determined. **Conclusion:** These data emphasize a causal role of CYP2E1 in the generation of εdA, in the fibrotic progression of ALD, and in alcohol mediated hepatocarcinogenesis. Thus, CYP2E1 may be a target in the treatment of ALD.

2.14 Cell death biomarkers are associated with outcome in hepatorenal cirrhotic patients

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Background and aim: Hepatorenal syndrome (HRS) is a severe complication of patients with liver cirrhosis and associated with high mortality rates if left untreated. Levels of cell death parameters are significantly elevated in patients with acute and chronic liver diseases. However, the role of these markers in patients with HRS is unknown. Aim of the study was to investigate if serum levels of M30 and M65 correlate with the risk of HRS development in cirrhotic patients and clinical outcome of HRS. **Methods:** Between April 2013 and July 2015, clinical relevant parameters from from patients with liver cirrhosis with HRS (66) and liver cirrhosis without HRS (49) were prospectively assessed. Serum samples were collected and serum levels of M30 and M65 were measured by ELISA. **Results:** Patients in the HRS group presented with more advanced stages of cirrhosis (Child-Pugh B: 17% C: 76%, $p =< 0.0001$). M30 and M65 significantly discriminated patients with HRS compared to non-HRS controls (631 U/l vs. 261 U/l, $p < 0.0001$; 1.097 U/l vs. 401 U/l, $p < 0.0001$). Within the group of HRS patients, higher levels of M30 and M65 were associated with higher MELD (M30: $p = 0.002$; M65: $p = 0.002$). Furthermore, higher levels of M30 and M65 were associated with poor response to terlipressin therapy (M30: $p = 0.009$; M65: $p = 0.007$). Overall survival was significantly shorter in the HRS subgroup with high levels of M30 and M65 (M30: $p = 0.04$; M65: $p = 0.03$). **Conclusions:** Serum levels of M30 and M65 are elevated in patients with liver cirrhosis and HRS and are associated with severity of liver disease as well as response to terlipressin therapy and overall survival. Cell death parameters could have potential value as a diagnostic and prognostic tool in patients with liver cirrhosis and HRS.

2.15 Development of autoantibodies against "rings and rods"-associated IMPDH2 in chronic hepatitis C genotype 1 infection during protease inhibitor based triple therapy in a "real life" cohort

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Background: Autoantibodies against inosine-5'-monophosphate dehydrogenase 2 (IMPDH2; "rings and rods" pattern) develop in chronic hepatitis C (CHC) patients under treatment with peg-interferon (IFN) and ribavirin (RBV), an inhibitor of IMPDH2. We investigated the influence of the alternative therapy with a regimen including direct acting antivirals (DAA)/ribavirin on anti-IMPDH2 autoantibody generation and the use of anti-IMPDH2 development as a marker for therapy outcome (sustained virologic response, SVR). **Patients and methods:** We analyzed a "real life" cohort of 104 unselected CHC genotype 1 (GT1) patients treated with IFN/1st-generation DAA/RBV prospectively compared to a historic cohort of 59 IFN/RBV treated CHC GT1 patients. 1st-generation DAA were boceprevir (BOC) or telaprevir (TPR). Serum autoantibodies were tested by indirect immunofluorescence (IFA) using recombinant IMPDH2 expressing HEK293 cells and native HEp2-cells as substrates. **Results:** 64/163 (39%) CHC patients turned anti-IMPDH2 positive during therapy, but only 43/163 (26%) showed also "rings and rods" structures. 99/163 (61%) were tested anti-IMPDH2 negative. 53/104 (51%) CHC patients undergoing IFN/DAA/RBV therapy were anti-IMPDH2 positive and 38/104 (37%) were in parallel anti-"rings and rods" positive. HCV clearance/SVR rate after IFN/DAA/RBV therapy and anti-IMPDH2 status were not significantly correlated. **Conclusion:** CHC GT1 patients treated with IFN/1st-generation DAA/RBV developed anti-IMPDH2 autoantibodies comparable to previous studies including patients under IFN/RBV therapy. Anti-IMPDH2 development promises no use as a marker for therapy outcome in CHC GT1 patients.

2.16 Dysfunction of hepatic regulatory T cells in experimental sclerosing cholangitis is associated with IL-12 signaling

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Background: Regulatory T cells (Treg) are essential for maintenance of immunological tolerance. Reduced numbers or function of Treg have been described for several autoimmune diseases including autoimmune liver diseases. Thus, we could recently describe a reduced number and possibly reduced function of regulatory T cells (Treg) in patients with primary sclerosing cholangitis (PSC). Therefore, Treg expansion might serve as a therapeutic approach. **Methods:** In this study Treg were expanded by repeated injection of IL-2/IL-2Ab complex in mouse models of cholangitis (Mdr2-/-, DDC) or colitis (DSS) as control. In vitro suppressive capacity and gene expression were analyzed in isolated hepatic and splenic Treg. **Results:** We observed a significant increase of Treg numbers

in the liver after enrichment of the endogenous Treg population via application of IL-2/anti-IL-2-complex (PBS: 4.6% vs. IL-2-K: 27.2%, p < 0.03). Tregs from PBS treated as well as IL-2/anti-IL-2-complex treated animals were mainly localized in the inflamed portal tracts (PBS: 640 vs. IL-2-K: 3780 Foxp3+ cells [pro mm2], p < 0.03). However, although Treg expansion was associated with reduced pro-inflammatory IL-17 (PBS: 160.8 vs. IL-2-K:103.7 [pg/ml], p < 0.03) and increased anti-inflammatory IL-10 production by hepatic lymphocytes, the severity of cholangitis was not reduced. This was in contrast to the suppression of colitis by Treg enrichment (PBS-Score 7.7; IL-2-K-Score 4.9, p < 0.02), suggesting a reduced functionality of intrahepatic Treg. Indeed, hepatic Treg manifested reduced Foxp3 expression and reduced suppressive capacity compared to splenic Treg. The reduced Foxp3 expression in Treg could be linked to increased IL-12 receptor beta 2 expression and IL-12 signaling. Accordingly, IL-12 Receptor beta 2 knockout mice (IL-12rb2-/-) were able to maintain hepatic Treg functionality. **Conclusion:** Hepatic Treg expanded in vivo improved the course of experimental colitis but failed to improve the course of cholangitis, which was related to the effects of hepatic IL-12 on Treg. Therefore, neutralization of IL-12 should be considered as part of treatment strategies to improve the efficacy of Treg treatment in liver diseases.

2.17 Increased in vivo and in vitro TH17 differentiation in patients with Primary Sclerosing Cholangitis

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Introduction: Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease. Immune dysregulation is considered to be part of disease pathogenesis. Recently, we could show that in PSC patients, IL-17-producing T cells are located around bile ducts and the in vitro stimulation of peripheral blood mononuclear cells (PBMC) with pathogens resulted in increased Th 17 responses. In this study, we further studied the role of Th 17 cells in PSC disease pathogenesis and progression and investigated underlying mechanisms leading to increased Th 17 responses. **Methods:** 69 patients with PSC, 37 patients with primary biliary cholangitis (PBC) and 45 healthy controls were included in this study. Disease staging was defined by clinical and imaging signs of liver cirrhosis. IL-17 production of blood derived CD 4+ T cells was analyzed by flow cytometry. IL-6 and IL-1β production from blood derived monocytes was analyzed by ELISA after in vitro stimulation with heat-inactivated C. albicans for 24 hours. For Th 17 differentiation assays blood derived naïve CD 4+ T cells were cultured for 12 days in the presence of IL-6, IL-1β, IL-23 and TGFβ. Th 17 differentiation was analyzed by flow cytometry. **Results:** Upon ex vivo stimulation of lymphocytes we observed significantly increased numbers of peripheral blood IL-17 producing CD 4+ T cells in PSC patients compared to control groups (PSC: 2.52% vs. Healthy: 1.67% vs. PBC: 1.61%, p = 0.0007). The increased frequencies of Th 17 cells in PSC patients correlated with advanced stage of disease (advanced PSC: 3.79% vs. PSC: 2.17% vs. Healthy: 1.70% vs. advanced PBC: 1.78% vs. PBC: 1.7%, p = 0.0006), indicating a potential role of IL-17 in disease progression. Monocytes from PSC patients produced significantly more IL-1β (PSC: 18901 pg/ml vs. Healthy: 14698, p = 0.046) and IL-6 (PSC: 46603 pg/ml vs. Healthy: 35903 pg/ml, p = 0.037) than healthy controls upon stimulation with C. albicans. Both cytokines are required for Th 17 cell differentiation. Additionally, in in vitro conversion assays we found that naïve CD 4+ T cells, isolated from PSC patients, showed an increased differentiation towards a Th 17 phenotype compared to control groups (PSC: 1.36% vs. Healthy: 0.41% vs. PBC:= 0.31%, p = 0.0002). **Conclusions:** Patients with PSC showed higher frequencies of Th 17 cells in peripheral blood and these frequencies were associated with disease stage. Monocytes from PSC patients produced more cytokines required for Th 17 differentiation and the ability of naïve CD 4+ T cells to convert into Th 17 cells in vitro. Together these results should stimulate further research into the pathogenetic role of IL-17 in this enigmatic liver disease.

2.18 Elastografie bei akutem Leberversagen: eine prospektive Studie

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Elastografiemessungen der Leber durch FibroScan (Echosens®) sind ein etabliertes Verfahren zur nicht invasiven Abschätzung der Lebersteifigkeit bei Patienten mit chronischen Lebererkrankungen. Der Stellenwert dieses Verfahrens bei Patienten mit akuter Leberfunktionsstörung bzw. Leberversagen (Acute Liver Injury (ALI), Acute liver failure (ALF)) ist bislang kaum evaluiert. In einer prospektiven Histologie-kontrollierte Kohorte-Studie untersuchen wir den Stellenwertes eines FibroScans als prädiktiven Parameter zu Differenzierung von Patienten mit ALI und ALF. Es wurden 40 Patienten (13 Männer; 27 Frauen; Medianes Alter 42,2 Jahre) ohne vorbestehende Lebererkrankung mit ALF (n = 6, mit hepatischer Encephalopathie (HE), INR > 1,5) bzw. ALI (n = 34, ohne HE, INR> 1,5) eingeschlossen. Bei allen Patienten erfolgten initiale Elastografie, laborchemische Untersuchungen, sowie Lebersonografien (40/40) einschließlich Duplex der Lebergefäße (26/40). Bei 80% Patienten (4/6 der ALF Gruppe bzw. 6/34 der ALI Gruppe) wurde zusätzlich eine Leberbiopsie in der initialen Phase durchgeführt. Eine Lebertransplantation war bei Spontanremission in keinem Fall notwendig. Valide Elastografie-Messungen konnten bei 28/40 Patienten erfasst werden. Die Median stiffness lag bei 11,3 kPa im Median über die gesamte Gruppe, 21,3 kPa in der ALF Gruppe und 10,9 kPa in der ALI Gruppe. Der Unterschied zwischen den beiden Gruppen verblieb jedoch ohne statistische Signifikanz. Die Höhe der Median stiffness über die gesamte Kohorte betrachtet korrelierte jedoch signifikant mit Lebersyntheseparametern (INR: r = 0,53; p = 0,01 bzw. Albumin: r = −0,59; p = 0,01). Ebenso zeigte sich eine signifikante Korrelation zu der Höhe des CRPs (r = 0,56; p = 0,01). Darüber hinaus war eine signifikante Korrelation zwischen dem Schweregrad der Leberparenchymnekrosen und der Höhe der Median stiffness (r = 0,62; p < 0,05) erkennbar. Eine Diskriminierung zwischen ALF und Ali ist in unserer Kohorte trotz deutlich erhöhter Messwerte im FibroScan nicht möglich. Eine signifikante Assoziation zwischen Median stiffness und Parametern der Inflammation, Lebersyntheseleistung sowie Schweregrad des Leberparenchymschadens rechtfertigen weitere Untersuchungen an größeren Kohorten mit ALF bzw. ALI.

2.19 ERCP in critically ill patients unit is a safe procedure and does not increase mortality

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Background: Endoscopic retrograde cholangiopancreatography (ERCP) is an important intervention in the management of biliopancreatic disease. Although there is a well-defined spectrum of complications, the procedure itself is considered to be safe in most patients. However, data on critically ill patients treated in intensive care unit (ICU) who undergo emergency ERCP are limited with regard to outcome and complication rate **Patients and methods:** A retrospective analysis was performed of 102 patients treated in intensive care undergoing a total of 121 ERCP between 2002 and 2016 at the University Hospital Essen. Indications, interventional success rates, survival, outcome and ERCP-related complications were analyzed. Patients' condition pre-ERCP was categorized by using the "Simplified Acute Physiology Score" (SAPS) 3 score, thus predicting in-hospital mortality. **Results:** 76 of 102 patients (74.5%) with an average SAPS 3 score of 67.5 (translating into a calculated probability of in-hospital mortality of 50%) were referred to ERCP from surgical intensive care units, the remaining 26 (25.5%) were treated for non-surgical disease ICU. The majority of patients were males (63.7%), the mean age was 54.1 [21 – 87] years. The indications for ERCP were: biliary leakage after major liver surgery (44.6%), cholestasis/cholangitis (38.0%), cholangiopathy after liver transplant (15.7%) and bleeding after prior ERCP (1.7%). The intervention was successfully performed in 92.6%. One patient (0.8%) died from septic shock during the procedure. Post-ERCP pancreatitis occured in 16.2%. The mortality of all patients was 52.2% (compared to a predicted mortality of 50%). **Conclusions:** ERCP is safe in critically ill patients treated on ICU without increasing procedure-associated complications compared to non-critical patients. The proce-

dure itself does not increase mortality in this challenging group of patients.

2.20 ERCP in patients with PSC and cirrhosis is not associated with an increased rate of complications

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Background: Primary sclerosing cholangitis (PSC) is a chronic inflammatory disease of intra- and extrahepatic bile ducts with progression to liver cirrhosis and ultimately the need for organ transplantation. Endoscopic treatment is recommended in patients with dominant stenosis and/or clinical symptoms such as pruritus or cholangitis. Whether ERCP is safe and effective in patients that have already progressed to cirrhosis is unknown. We aimed to assess efficacy and complications in a large PSC cohort from a single centre. **Methods:** Out of 380 patients with PSC, 208 patients were treated endoscopically between the years 1999 and 2016. We identified 62 patients who had already developed liver cirrhosis at the time ERCP and another 146 patients with PSC without cirrhosis. Mean age was similar in both groups (47,4 vs. 44.8 years). A total of 592 ERCP procedures were analysed, with 186 ERCP procedures in the cirrhotic patients and 406 ERCP procedures in patients without cirrhosis. Data were analyzed retrospectively. As procedure related complications we considered acute pancreatitis, cholangitis, bleeding (requiring blood transfusion or re-intervention) and perforation (defined as bile duct injury requiring re-intervention, stenting, surgery or prolongation of hospital stay). **Results:** Overall 43 (7.2%) procedure related complications were documented. Complications were slightly more frequent in the group without cirrhosis (5.4% vs. 8.1%). In patients with liver cirrhosis, we identified 10 complications including three patients with post-ERCP pancreatitis (2%), four patients developed post-ERCP cholangitis (2,1%), two perforations (1%) and one bleeding (0.5%). There were no operations needed. In the group of PSC patients without liver cirrhosis, 33 complications were noted (8.1%), including 15 patients with post-ERCP pancreatitis (3,7%), 10 patients with post-ERCP cholangitis (2%), 6 perforations (1.4%) and 2 bleedings (0.5%). One operation with drainage of abdominal bile collection was needed to manage this patient. Rate of sphincterotomy during ERCP was similar in both groups (24% vs. 23%). **Discussion:** Endoscopic therapy in patients with PSC and progression to liver cirrhosis was not associated with a higher rate of complications. Therefore cirrhosis should not preclude ERCP intervention if needed.

2.21 Evaluation of TTR toxicity in supernatants of FAP-derived hepatocyte-like cells

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Familial amyloid polyneuropathy (FAP) is a neurodegenerative disease caused by mutations of the transthyretin (TTR) gene, which is primarily secreted by the liver. Mutations in TTR cause misfolding of the protein ultimately leading to extracellular deposition of amyloidogenic TTR in tissues and organs, disrupting regular function. Typically, amyloid deposits are observed in biopsies of FAP patients. Due to the variety of the clinical findings and the rare incidence of the disease, many patients can only be diagnosed several years after onset of the disease. Besides genetic testing of TTR there is no single biochemical assay to confirm FAP diagnosis. In vitro, purified TTR derived from E. coli was reported to be toxic when incubated with tissue culture cells. We addressed the question whether supernatants derived from hepatocyte-like cells (HLCs) differentiated from induced pluripotent stem (iPS) cells of FAP patients induce cellular toxicity on the IMR-32 neuronal cell line. After three to seven days of incubation, the cell viability was determined by an MTT (3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide) assay and the respective absorbance was determined. TTR protein levels were determined by ELISA and quantified by western blot analysis. Furthermore, stability of TTR was assessed by treatment with the small-molecule tafamidis. Our preliminary data indicate that supernatants of FAP patients exhibit toxicity that is related to variant TTR in contrast to supernatants derived from other cell lines. The assessment of toxicity observed in supernatants of FAP-HLCs could be valuable to support the tedious diagnostic evaluation of patients to monitor efficacy of treatment as well as to investigate TTR-related amyloidogenesis.

2.22 Extracorporeal blood purification improves nasobiliary drainage (NBD)-refractory pruritus in a BRIC type 2 patient

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Introduction: Benign recurrent intrahepatic cholestasis type 2 (BRIC2) is a rare genetic disease caused by mutations of the hepatobiliary transporter for bile salts (ABCB11). It is characterized by episodes of cholestatic itch and jaundice. Here we present a case of refractory BRIC type 2 who improved rapidly with plasma separation and anion absorption therapy. **Case presentation:** The 23-year-old male patient, a compound heterozygous carrier of the ABCB11 mutations c.3491delT and c.3826C>T, was referred to our department with a prolonged episode of refractory pruritus due to BRIC type 2. It was his 4th BRIC episode and it had lasted for several weeks before admission to our department. Previously he had undergone therapy with ursodeoxycholic acid, rifampicin and nasobiliary drainage (NBD), none of which led to sustained improvement of pruritus. At admission his serum bilirubin concentration was 27.6 mg/dl and AP activity was 342 U/l (normal range < 129 U/l) but GGT was normal, consistent with BRIC type 2. He suffered from pruritus intensity 7/10 points on the visual analog scale (VAS). Transiugular liver biopsy confirmed bland cholestasis but neither cirrhosis nor vanishing bilie ducts. Given the refractory pruritus, we started extracorporal blood purification with plasma separation and anion adsorption. This resulted in an improvement of pruritus already on the first day (VAS decrease to 4). During the 4-month course of this therapy his pruritus decreased to < 1 and bilirubin was < 2.0 mg/dl. Currently he is symptom-free and blood tests do not indicate cholestasis. **Discussion:** Invasive techniques, namely NBD or extracorporal blood purification, are known to relieve refractory cholestatic pruritus. BRIC2 is caused by dysfunction of the hepatocanalicular bile salt export pump, causing cessation of bile salt-dependent bile flow. Hence we reckon that NBD placement fails to improve cholestasis in this genetically defined subgroup of BRIC patients, and we recommend extracorporal blood purification in this situation instead.

2.23 First time use of PRISM, an easy to use and suitable tool to characterize burden of disease, in patients suffering from chronic Hepatitis C: Results from the German observational study LIFE-C

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Background: LIFE-C is a non-interventional observational study in patients with chronic Hepatitis C (CHC) receiving Ombitasvir, Paritaprevir and Ritonavir ± Dasabuvir ± Ribavirin. Here, we report the burden of disease at baseline by analyzing the self-illness-separation (SIS) determined by Pictorial Representation of Illness and Self Measure (PRISM). **Methods:** Baseline demographics (incl. PRISM, Functional Assessment of Chronic Illness Therapy fatigue subscale [FACIT-F], Work Productivity and Activity Index [WPAI:Hepatitis C V2.1], Patient Activation Measure [PAM-13]) were recorded. Correlation between SIS and continuous patient characteristics was investigated by linear regression analysis and associations with categorical characteristics by analysis of variance. **Results:** 104 patients enrolled until March 31st were analysed (Table 1). The median SIS [n = 100] was 11.9 cm (IQR: 4.6 – 19.4). SIS correlated weakly with FACIT-F scores (Pearson correlation coefficient [PCC] = 0.3926 [p = 0.0003]) and total activity impairment (PCC = -0.4021

[p = 0.0002]), but no further variables, e.g. BMI. Significantly higher SIS was associated with age > 65 years (Median SIS: 18 – 65 years [n = 81]: 10 cm, 66 – 84 years [n = 19]: 17.5 cm, p = 0.0061) and the presence of co-infections (HIV [n = 8] or HBV [n = 1]; median SIS: present [n = 9]: 17.0 cm, absent [n = 91]: 11 cm, p = 0.0364), but no association was detected with other characteristics, e.g. sex, race, or viral load. **Conclusion:** SIS correlated weakly positively and negatively, respectively, with FACIT-F scores and total activity impairment due to hepatitis. These weak correlations may be due to its high variability. The median score for SIS is within the range observed for Diabetes (1). PRISM is an easy to use and apparently suitable tool to determine the burden of disease in CHC patients. Data of patients recruited until October 2016 will be presented. **Reference:** [1] Klis S et al. Health Qual. Life Outcomes 6, 104 (2008).

2.24 HCC prognostic signatures are not unique

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Throughout the past decade, multiple molecular signatures predicting the course of Hepatocellular carcinoma (HCC) were reported. However, these signatures have not yet been integrated into clinical routine decision making. Investigating randomly chosen signatures of various size, we demonstrate that approximately 15.7 to 30.1% of all random signatures exhibit prognostic potential. This finding was further confirmed by using predefined, functionally related gene sets such as GSEA, KEGG pathways or GO terms. Given the large number of potentially prognostic random signatures, we further investigated whether combining multiple signatures would result in an improved significance and stability of prognostic capability. These combined analyses demonstrated that HCC patient samples may be divided into high predictable and low predictable samples. In high predictable samples most random signatures resulted in comparable prognosis. In turn, we demonstrate by training and independent validation, that if most random signatures show the same prognosis, this result must be considered highly reliable. Thus, using "swarm intelligence" for prognostic evaluation of HCC may be superior to single prognostic signatures of any size and increase stability of prognosis.

2.25 Hepatitis E seroprevalences in pregnant and non-pregnant women in a southern region of Brazil

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Hepatitis E was known to be a disease occurring mainly in Asian and African countries and to cause liver failure in pregnant women. A variety of different transmission modes were characterized in recent years. Genotype 3 infection has been recognized in industrial countries with severe chronic infections in immunocompromized individuals and serious HEV-associated autoimmune disorders. The anti-HEV prevalence and HEV genotype distributions in South America are not well defined. We aimed to study risk factors and the seroprevalence of hepatitis E in a group of pregnant women in Brazil. **Methods:** A group of 209 pregnant women and 199 female blood donors, paired by age, were recruited from 2002 to 2003, in Clinics Hospital, Federal University of Paraná, Brazil. A broad questionnaire evaluating risk factors was applied. The serum was kept frozen at -80 °C since then and analyzed in Germany in 2014 where the samples were tested for anti-HEV IgG (Wantai – ELISA assay), anti-HBc, HBsAg and Anti-HCV, sponsored by the DZIF HepNet Study-House. HEV PCR was tested by an in house method. **Results:** A total of 40 (19%) pregnant women and 51 (26%) female blood donors tested positive for anti-HEV IgG. None of the women tested positive for HEV RNA. Anti-HEV seropositivity was associated with age but not with history of blood transfusion, ethnicity or income. In total four (2 in each group) samples were tested HBsAg positive (0.96%), 25 pregnant women showed an anti-HBc reaction (11.9%), compared to 3% (n = 2) blood donors. No correlation could be detected between anti HBc and anti HEV positivity. None of the women tested positive for anti HCV. **Conclusion:** This is the first report on HEV seroprevalence rates in pregnant women and female blood donors in a Southern Region of Brazil using the Wantai assay. The overall prevalence of anti-HEV was comparable to western and central European countries. Importantly no sample tested positive for HEV RNA indicating that acute HEV infection is not a major threat for pregnant women in Brazil. The lack of association with other viral hepatitis markers, income, education degree and ethnicity suggests that HEV is transmitted beyond socioeconomic barriers. Furthermore eating habits are not included in this study and may play a role. More studies are needed to determine the health burden associated with this infection.

2.26 Improvement of IgG4-associated autoimmune cholangitis in a patient with HCV-cirrhosis by treatment with sofosbuvir and ledipasvir: a case report

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Background: Hepatitis C virus (HCV) causes not only liver disease that may contribute to cirrhosis and liver failure but also can cause extrahepatic manifestations in nearly 75% ranging from cryoglobilinaemia to lymphoma. As a new clinical entity IgG4-associated autoimmune cholangitis (AIC) has gained attention characterized by elevated serum IgG4-level and diffuse cholangiographic abnomalities that respond well to steroid therapy. **Case report:** A 85-year old female patient with IgG4-associated AIC and HCV GT 1b cirrhosis was sucessfully treated with sofosbuvir (SOF) and ledipasvir (LED). Viral load was below detection after 4 weeks of treatment. SVR 24 has been achieved. Intriguingly, IgG4-levels decreased by the antiviral treatment from over 1'2750 mg/l to 5728,6 mg/l within 4 month. Patient characteristics, data on lab results, and the course of disease and treatment were documented from November 2014 to September 2015. **Conclusion:** This is the first reported case of IgG4-related AIC improving by antiviral treatment of HCV. Even if the pathogenetic factors of IgG4-associated AIC remains not fully understood, these finding support the assumption that inflammatory processes, in this case the chronic active HCV infection, implicated the IgG4-related disease and antiviral treatment leads to remission.

2.27 Lack of Polarity in liver progenitor cell-derived hepatocytes determines poor clinical outcome of acute-on-chronic liver failure

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Background & Aim: Submassive hepatic necrosis (SMHN) is the defining histological feature of acute-on-chronic liver failure (ACLF). In the areas of SMHN, liver progenitor cells (LPC) critically contribute to liver regeneration. This study investigated the key events that determine LPC-mediated liver regeneration in ACLF following SMHN. **Methods:** Liver tissues from 53 ACLF patients (50 receiving liver transplantation (LT) vs. 3 spontaneously recovered) were investigated by immunohistochemistry for Cytokeratin 7 and CD26 to identify LPC/intermediate hepatocyte-like cell (IHLC) and hepatocyte polarity. Transcription factor gene profile was analyzed by microarray. Canaliculi were further examined in collagen sandwich-cultured mouse hepatocytes. **Results:** Rapid ductular reaction was observed in all ACLF patients receiving LT even if their clinical dura-

tion were 2 days. In these patients, LPC differentiated into IHLC and hepatocytes with time, whereas liver function further deteriorated over time. Notably, LPC-derived hepatocytes did not form canaliculi. In contrast to the patients receiving LT, hepatocytes of recovered ACLF patients displayed intact polarity with formation of canaliculi. Microarray analyses and immunohistochemistry revealed remarkably reduced HNF4α expression in liver tissues from the ACLF patients receiving LT. In contrast, hepatocytes in recovered ACLF patients demonstrated strong HNF4α expression. However, in vitro study showed that disruption of HNF4α expression did not impact hepatocyte polarity. **Conclusion:** Recovery from ACLF is associated with formation of canaliculi and strong HNF4αα expression. HNF4α might play a critical role in regulating LPC differentiation towards hepatocytes. Uncovered factors determine polarity with canaliculi formation and function of LPC-derived hepatocytes that decide the clinical outcome of ACLF.

2.28 miR-1224 is upregulated in hepatic ischemia-reperfusion injury and induces cell death via Sp1/Nfib inhibition

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Hintergrund: MicroRNAs (miRNAs) sind direkt an der Regulation von Genexpressionsmechanismen beteiligt. Im Rahmen der Lebertransplantation kommt es regelmäßig zu Ischämie/Reperfusions (I/R)-bedingtem Leberschaden, was ein wichtiges klinisches Problem darstellt. Es stehen jedoch nur wenige Daten zur Rolle von miRNAs im I/R-Leberschaden verfügbar. Es sollte daher die Funktion von miRNAs im Kontext des I/R-Schadens funktionell untersucht werden. **Methoden:** PCR-basierte miRNA-microarray Untersuchungen wurden an Leberproben von Mäusen nach Induktion eines I/R-Leberschadens und Kontrollmäusen durchgeführt. Die Resultate wurden in menschlichen Proben mittels PCR validiert und mit klinischen Parametern korreliert. Der Effekt der miR-1224 wurde durch Transfektionsversuche untersucht und Zielgene identifiziert sowie funktionell im Rahmen der I/R charakterisiert. **Resultate:** Es zeigte sich in den arraybasierten Genexpressionsanalysen dass die miR-1224 zu einer Gruppe von im Rahmen der I/R differentiell regulierten miRNAs gehörte. Die miR-1224 war sowohl in der Leber als auch um Serum von Mäusen und auch Menschen nach I/R Leberschaden hochreguliert und korrelierte mit Markern des Leberzellschadens. Darüber hinaus zeigten primären Hepatozyten und Leberzelllinien nach Behandlung mit H2O2 eine signifikante Mehrexpression der miR-1224. Auf funktioneller Ebene führte die artifizielle Überexpression der miR-1224 zu vermindertem Zellüberleben, was auf verminderte Zellproliferation und Induktion von caspase 3/8 -vermittelter Apoptose zurückgeführt werden konnte. Mittels reporter assays wurden die Transkriptionsfaktoren Sp1 und Nfib als Zielgene der miR-1224 identifiziert. Die Inhibition dieser Gene führte zu einem vergleichbaren Effekt wie die Transfektion der miR-1224 im akuten Leberschaden. Schließlich zeigte sich konsistent zur Mehrexpression der miR-1224 in den unterschiedlichen Modellen des Leberzellschadens eine Mehrexpression dieser Zielgene. **Schlussfolgerung:** Im Rahmen der I/R induzierten Leberschädigung kommt es zu einer Induktion der miR-1224 Expression. Dies führt über eine Minderexpression von Sp1 und Nfib zu vermehrtem Zelltod und verminderter Zellproliferation.

2.29 Nuclear ErbB2 Expression of Hepatocytes is Correlated with Distinct Histopathological Features

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Background: ErbB2 is a prominent member of the epidermal growth factor receptor superfamily, a group of transmembrane receptors that mediate proliferation and cell growth in many tissues and are frequently investigated as oncogenic drivers and therapeutic targets in cancer. Besides transmembrane signaling, ErbB2 may also translocate into the nucleus and mediate distinct nuclear signaling effects, including DNA repair and cell cycle arrest. We recently demonstrated nuclear ErbB2 expression in hepatocytes, but it is still unclear, which disease conditions result in hepatocellular ErbB2 expression. **Methods:** We analyzed 674 liver core needle biopsies from patients with hepatic dysfunction for immunohistochemical ErbB2 expression and correlated expression intensity with histopathological diagnosis and several histomorphological parameters: grades of fibrosis, steatosis, inflammation, cholestasis, occurrence of hepatocellular ballooning and Mallory-Denk-bodies. **Results:** Hepatocellular ErbB2 positivity significantly correlated with increasing fibrosis grade and also with grade of chicken wire fibrosis. Furthermore, ErbB2 positivity correlated with inflammatory activity of steatohepatitis with particularly frequent and strong ErbB2 expression in alcoholic steatohepatitis compared to nonalcoholic steatohepatitis. Other hepatitides like viral hepatitis or autoimmune hepatitis were mostly ErbB2 negative. Interestingly, in case of steatohepatitis ErbB2 positivity was associated with low grade of steatosis, whereas in inflammation-free liver steatosis ErbB2 positivity was not correlated with grade of hepatocellular steatosis. The histopathological finding of cholestasis (i.e. bilirubinostasis and cholate stasis), independently of disease background, frequently was accompanied with hepatocellular ErbB2 expression, too. Moreover, ErbB2 positivity was significantly associated with hepatocellular ballooning and also with occurrence of Mallory-Denk-bodies. **Conclusion:** Hepatocellular ErbB2 expression is correlated with several histomorphological features of hepatocellular dysfunction and damage like fibrosis, chicken wire fibrosis, cholestasis, hepatocellular ballooning and inflammatory activity of steatohepatitis. Of note, inflammation-associated hepatocellular ErbB2 expression was observed only in steatohepatitis, not in viral hepatitis or autoimmune hepatitis, although these hepatitides come along with significant hepatocellular damage, too. However, ErbB2 positivity is not only a result of alcoholic liver injury, but also occurs in diverse cholestatic liver conditions. Though, ErbB2 positivity may also serve as additional tool in histopathological differential diagnosis of liver disease. Moreover, further mechanistic studies on the role of ErbB2 expression on hepatocyte's fate have to be conducted in future.

2.30 Postmortal diagnosis of hemophagocytic lymphohistiocytosis in a patient with liver failure of unknown etiology

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Case: A 68 year old man was transferred to our transplant center due to liver failure of unknown etiology. Initial elevated liver values have been documented three weeks before. The patient reported on increased fatigability, night sweats and weight loss of 16 kilogram during the past five month. Apart from a Hepatitis A infection during childhood hepatologic medical history was unremarkable. MELD score was 28 on admission. Acute on chronic liver failure was suspected due to results of diagnostic workup. Criteria for high urgent liver transplantation were not met. Diagnostic workup did not reveal any conclusive results. **Course of disease and diagnosis:** Despite supportive therapy the patient's condition detoriated rapidly. Different complications arose. Twelve days after admission to our hospital the patient was transferred to the ICU after cardiopulmonary resuscitation, where he died of rapidly progressive disease. Autopsy revealed lymphohistiocytosis and hemophagocytosis in bone marrow and perihepatic lymph nodes. Histopathological examination of the liver showed uncharacteristic moderate portal and periportal hepatitis together with liver cell necrosis, venoocclusive disease and periportal to septal fibrosis. Considering all clinical and diagnostic findings the patient meets five out of eight diagnostic criteria for hemophagocytic lymphohistiocytosis (HLH). So HLH might be considered as cause for liver failure in our patient. An inciting event could not be identified retrospectively. **Conclusion:** HLH is a rare cause of liver failure in adults and might be associated with a fulminant course of disease. Therefore diagnosis of HLH should be considered as rare cause of liver failure in patients with liver failure of unknown etiology.

2.31 Ultrasound diagnosis of hepatic steatosis represents increased metabolic risk

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Background and Aims: Hepatic steatosis is the basis of non-alcoholic fatty liver disease (NALFD). Mere fat accumulation within hepatocytes is considered the mild form of NAFLD, but can progress in some patients to

advanced steatohepatitis (NASH), which may lead to fibrosis, cirrhosis or hepatocellular carcinoma. However, even hepatic steatosis alone may be a risk factor for cardiovascular disease (CVD). **Patients and Methods:** In the present real life study 106 patients from the outpatient clinic of the Department for Gastroenterology and Hepatology with either NAFLD (n = 60) or other typical diagnosis (n = 46) were included. Ultrasound examination identified 77 patients with hepatic steatosis. Liver enzymes, lipid profile, surrogate cell death markers, and adiponectin were determined. Transient elastography (Fibroscan®) and bioelectrical impedance analysis (BIA) were performed. **Results:** Mean patient age was 46 years (23 – 62) for non-NAFLD and 53 years (18 – 71) for the NAFLD group. ALT and AST did not differ significantly between the two groups. Adiponectin and HDL were significantly lower in NAFLD ($p < 0.05$) and BIA profiles showed higher fat and fat free mass. Non-NAFLD patients with steatosis also exhibited an adverse metabolic profile. Overall steatosis was associated with factors of metabolic syndrome (MS) and CVD. Prevalence of CVD and factors of MS hint to steatosis as an early event for these conditions. **Conclusion:** Patients with steatosis are at higher cardiovascular and metabolic risk without differences in transaminases levels compared to those without steatosis. Steatosis diagnosed by ultrasound needs to rise attention for further metabolic alterations including CVD.

2.32 Prädiktion fortgeschrittener Fibrose bei Patienten mit nicht-alkoholischer Fettleber (NAFLD) – Vergleich von NAFLD fibrosis score (NFS), Fibrosis-4 (Fib-4) score und APRI

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Einleitung: Die Inzidenz und Prävalenz der nicht-alkoholischen Fettlebererkrankung (NAFLD) sind in den westlichen Industrienationen stark zunehmend. Insbesondere Patienten mit fortgeschrittener Fibrose haben ein hohes Risiko des Progress der Erkrankung und eine erhöhte Mortalität. Zur Identifikation von Patienten mit fortgeschrittener Fibrose wurden neben Ultraschall-basierten Techniken auch nicht invasive Scores entwickelt, die klinische und laborchemische Merkmale kombinieren. Hierzu zählen der fibrosis-4 (Fib-4) score und der AST/Thrombozyten-Ratio-Index (APRI). Die deutsche S2K-Leitlinie zur NAFLD empfiehlt den NAFLD-fibrosis score (NFS) zur Identifikation von Patienten mit fortgeschrittener Fibrose vor einer Leberbiopsie. **Methoden:** 365 NAFLD-Patienten, welche sich zwischen 2012 und 2016 in der Universitätsmedizin Mainz vorstellten, wurden in die prospektiv geführte Patientenkohorte aufgenommen. Die aktuelle Auswertung bezieht sich auf 110 dieser Patienten mit erfolgter histologischer Untersuchung. **Ergebnisse:** Insgesamt wurden 110 Patienten mit einem mittleren Alter von 48,2 Jahren in die Analyse eingeschlossen. 51,8% waren männlich. Die untersuchten Patienten hatten einen hohe Frequenz an metabolischen Risikofaktoren: 43 (39,1%) Patienten litten an einem manifesten Diabetes mellitus, 33 (30%) waren übergewichtig (BMI 25 – 30 kg/m2) und 63 (57,3%) adipös (BMI > 30 kg/m2), 73 (66,4%) hatten eine arterielle Hypertonie und 60 (54,5%) wiesen eine Dyslipidämie auf. Die Kriterien des metabolischen Syndroms (nach NCEP-ATP-III 2009) erfüllten 85,5%. Histologisch zeigte sich bei 13 der untersuchten Patienten ein hoher NAS Score (11,8%, NAS-score ≥ 5), wohingegen 97 (88,2%) einen NAS Score von < 5 aufwiesen. Bei 15 bestand eine Leberzirrhose (13,6%) und 6 Patienten hatten einen Normalbefund in der Leberhistologie. 36 (32,7%) Patienten hatten einen NFS ≥ 0,676 im Sinne einer hohen Wahrscheinlichkeit für fortgeschrittene Fibrose. Davon wiesen 21 Patienten eine fortgeschrittene Fibrose auf, entsprechend einem PPV von 58,3%. In der Gruppe mit intermediärer Wahrscheinlichkeit oder niedriger Wahrscheinlichkeit (NFS < 0,676) hatten 11 eine fortgeschrittene Fibrose. Der NPV lag bei 85,1%. 15 (13,6%) wiesen einen Fibrosis-4-score > 3,25 – definiert als Hochrisiko-Gruppe für fortgeschrittene Fibrose – auf. Der PPV lag bei 80%, der NPV bei 78,9%. Ein APRI-Score > 0,7 im Sinne einer fortgeschrittenen Fibrose lag bei 53 (48,2%) Patienten vor. Der PPV lag bei 45,3% und der NPV Wert bei 86%. **Schlussfolgerung:** Im Vergleich zur Literatur war der PPV des NFS zur Vorhersage fortgeschrittener Fibrose in der Hochrisikogruppe nur 58,3% und der NPV in der Gruppe mit niedriger und intermediärer Wahscheinlichkeit einer fortgeschrittenen Fibrose 85,1%. Im Vergleich der Scores wies der APRI-Score den höchsten NPV auf. Die Indikation zur Leberbiopsie erscheint über die untersuchten Scores hinaus im Kontext der klinischen Beurteilung sinnhaft zur Identifikation von Patienten mit fortgeschrittener Fibrose.

2.33 Regulation of p53 – a possible bacterial defense mechanism leading to epithelial barrier destabilization in spontaneous bacterial peritonitis?

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Background: Spontaneous bacterial peritonitis (SBP) is a life-threatening complication in advancing liver cirrhosis. SBP represents a bacterial infection of ascitic fluid without an intra-abdominal source of infection that could be treated surgically. Translocation of intestinal bacteria or bacterial products from the gut to mesenteric lymph nodes is crucial for SBP, with Escherichia coli (E. coli), Klebsiella pneumoniae, enterococci and streptococci being the most common germs. As soon as a SBP is suspected, patients must be treated with antibiotics. In this context, biomarkers for early SBP diagnosis are in the focus of interest, as they are not available so far. With regard to the development of early recognition systems and therapeutic concepts, pathomechanisms and signaling pathways of bacterial translocation in SPB were explored. **Methods:** To investigate effects of intestinal bacteria on epithelial cell junctions, monolayers of human intestinal epithelial cell lines Caco-2 (p53 deficient) and HCT-116 (p53 wildtyp) were cocultured with E. coli at day 5 to 8 post confluence. Infection with E. coli was performed with different MOI (0 – 10) for 40 – 240 minutes. Western Blot analysis was used to analyze changes in intracellular protein levels of Occludin, E-cadherin and the p53 family. **Results:** E. coli stimulation of HCT-116 cells resulted in a strong decrease of the tight junction protein Occludin, the adherens junction protein E-cadherin, and, remarkably, also p53. Consistently, following E. coli stimulation Caco-2 cells displayed reduced protein levels of Occludin and E-cadherin. However, in p53-deficient Caco-2 cells this reduction was less distinct compared to p53-wildtyp HCT-116 cells. **Conclusion:** These results highlight destabilizing effects of E. coli on intestinal cell junctions. Of specific clinical relevance, a regulation of the tumor suppressor p53 by E. coli was demonstrated. With a more distinctive downregulation in HCT-116 epithelial cells, we hypothesize an involvement of p53 in the regulation of epithelial permeability during bacterial infection. Reduced p53 levels in conjunction with destabilized intestinal epithelial integrity following bacterial infection might represent a mechanism to protect bacteria from intestinal immune responses and therefore to promote bacterial translocation in SBP.

2.34 Screening for the INCA trial: Increased prevalence of common NOD2 risk variants in patients with cirrhosis in tertiary liver centers

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Background and Aim: Patients with liver cirrhosis display an increased risk of bacterial infections that decrease survival rates. Nucleotide-binding oligomerisation domain containing 2 (NOD2) germline variants were found to be associated with spontaneous bacterial peritonitis and mortality in patients with cirrhosis (Appenrodt et al. 2010). The INCA (Impact of NOD2 genotype-guided antibiotic prevention on survival in patients with liver Cirrhosis and Ascites, EudraCT 2013 – 001626 – 26) trial investigates in a randomized double-blind, placebo-controlled design (norfloxacin 400 mg once daily vs. placebo) whether survival of a genetically defined high-risk group of patients with cirrhosis carrying NOD2 variants is improved by primary antibiotic prophylaxis. The primary endpoint is overall survival after 12 months of treatment. As the overall prevalence of NOD2 variants in patients with liver cirrhosis is unknown, our aim now was to analyze the prevalence of the NOD2 variants in patients being evaluated or screened for the INCA trial. **Patients and methods:** Patients for this national multicenter study are recruited in 17 centers throughout Germany. In the participating tertiary care liver centers, potential study participants are genotyped for the three common NOD2 variants (p.R702W, p.G908R and c. 3020insC) using a PCR-based assay with 5'-nuclease and fluorescence detection. We determined allele and genotype distributions for the NOD2 variants across the INCA study centers. **Results:** Overall, 1,646 patients have been genotyped for the three common NOD2 risk variants since February 2014. The frequency of any NOD2 risk variant was 22.3% (367/1646) for all centers, and for centers screening a minimum of 20 patients, the mean prevalence was 21.0% (SEM ± 1.2%, range 15 – 29%). More than 30 patients

were screened in Homburg (732), Bonn (103), Frankfurt (100), Hamburg (74), Jena (39), Kaiserslautern (76), Leipzig (35), Mainz (31), Heidelberg (47), Mannheim (137) and Halle (220). The most common risk variant was p.R702W (13.1%, 215 patients), followed by c. 3020insC (7.9%, 130 patients) and p.G908R (3.5%, 58 patients). Nineteen patients (1.2%) carried two risk variants. According to available data in healthy adults, the NOD2 variant allele frequencies in the ExAC browser (Europeans) are 3.5% (p.R702W), 2.0% (c. 3020insC), 1.5% (p.G908R), and combined 7.0%; in another cohort including healthy Caucasians, the prevalence rates were 4.3% (p.R702W), 2.3% (c. 3020insC), 1.2% (p.G908R), and combined 7.8% (Hugot et al. 2007). **Conclusions:** In our patients genotyped in tertiary care liver centers, the prevalence of NOD2 risk variants is about three times the frequency compared to the healthy population, with similar distribution of the three single variants. Investigation of the association of NOD2 risk variants with clinical endpoints is warranted.

2.35 Selective Endothelin-A-Blocker decrease portal pressure in patients with cirrhosis. A feasibility study combining the local intraarterial and systemic administration

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Increased levels of the vasoconstrictor Endothelin-1 (ET) in the intrahepatic circulation in cirrhosis is one of the main mechanisms involved in the development of portal hypertension. ET is acting through two different receptors (ET-A and ET-B), in which the ET-A receptor is the responsible one for vasoconstriction. BQ123 and Ambrisentan are selective ET-A receptor blocker. The aim of the study was to investigate, if the selective blockage of the Endothelin-A receptor is decreasing portal pressure and influencing hepatic arterial blood flow. **Methods:** In 12 patients with cirrhosis (Child-Pugh B/C 7/5; CP points 9.8 ± 1.3) hepatic arterial (HA) catheterization using a 5-F-catheter was performed and a Doppler flow wire introduced for continuous measurement of HA blood flow (HABF) (Hepatology 2003;37:385). BQ 123 (Clinalfa, Switzerland, dose intervals: 300, 500, 1000, 2000 nmol/l) was selective intrahepatic administered by arterial infusion. HABF and hepatic venous pressure gradient (HVPG) were measured before and during the infusion of BQ 123. In a second set of cirrhotic patients (n = 14; Child-Pugh A/B/C 4/6/4; CP points 7.9 ± 1.8) oral administration of ET-A blocker (Ambrisentan; Volibris, GlaxoSmithKline, Germany) was given. HVPG and Doppler hepatic arterial resistance index (PI and RI) were measured before and 90 minutes after administration. ANOVA for repeat measurements and Wilcoxon Test was used as statistical test. **Results:** BQ 123 caused vasodilatation in the hepatic artery (Δ% HABF ± SD: +176 ± 359%, p = 0.029). There was a trend towards a higher hepatic arterial vasodilatation in Child B (+238 ± 473%) compared to Child C (+88 ± 47%) patients. Infusion of BQ 123 leads to a decrease of HVPG (Δ%: -18 ± 26%, p = 0.048). Ambrisentan caused significant decrease of HVPG (-5.4 ± 6.8%, p = 0.001). The decrease of HVPG was higher (-7.0 ± 6.0%) with 10 mg Ambrisentan compared to 5 mg (-4.8 ± 7.6%). Hepatic arterial PI and RI were unchanged due to Ambrisentan administration. **Conclusion:** Selective intrahepatic arterial infusion of an Endothelin-A-Blocker increases hepatic arterial flow and decreases portal pressure. Both, the selective intrahepatic as well as the systemic administration of ET-A blocker decreased portal pressure. Inhibition of the Endothelin-A receptor might be beneficial in the treatment of portal hypertension.

2.36 Serum Zn Levels in Egyptian Patients with HCV Induced Chronic Liver Diseases: Evaluation and Clinical Significance

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HCV infection is a major health problem worldwide, In Egypt the estimated prevalence is about 22%. As Zinc (Zn) is the second most prevalent trace element in the body, the current study aimed to evaluate serum Zn levels in patients with HCV induced chronic liver diseases and to study the relationship between these levels and clinical profiles, histopathological criteria and HCC characters of the studied cases. Sixty nine patients aged from (18 to 67) years were included in addition to 23 age- and sex-matched healthy subjects serving as a control, all were stratified into three groups, G 1 included 23 patients with biopsy proven CH. G 2 included 23 patients with cirrhosis. G 3 included 23 patients with HCC on basis of abdominal ultrasonography, triphasic spiral CT Scan and AFP. Group 4 included 23 healthy persons serving as control group. All subjects underwent routine investigations and serum Zn levels were analyzed on atomic absorption spectrophotometer, meanwhile cirrhotic subjects were assessed for severity of disease by Child-Pugh classification. At presentation Serum zinc levels were significantly lower in chronic hepatitis group than control group on one hand and HCC group on the other hand ($p < 0.001$) and they were significantly decreased in Child class C patients than Child class A ($p = 0.023$). Significant positive correlation was found between serum Zn levels and age in cirrhotic group moreover, there was no significant correlation between serum Zn levels and any of laboratory parameters in the studied groups and fibrosis stages of chronic hepatitis group. Negative non significant correlation was detected between serum Zn levels and tumor multiplicity and BCLC in HCC group. From the present study we can conclude that serum zinc levels decreased significantly in chronic HCV infected patients and these levels decreased by increasing severity of liver disease according to Child classification. It is recommended to evaluate the role of zinc supplementation in treating clinical manifestation of liver cirrhosis and liver cell failure associated with HCV.

2.37 Six weeks of Sofosbuvir/Ledipasvir treatment of acute hepatitis C Virus genotype 1 monoinfection: Final results of the German HepNet Acute HCV IV Study

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Background: Early treatment of acute hepatitis C virus (HCV) infection with interferon alfa monotherapy is highly effective but is associated with frequent unfavorable side effects. There is no fully published study yet exploring the safety, efficacy and required treatment duration of interferon free treatment of acute hepatitis C virus monoinfection. Preliminary reports suggested that ledipasvir/sofosbuvir therapy is effective in acute hepatitis C but relapses were reported in HIV-coinfected patients after 6 weeks of treatment. **Methods:** The German HepNet Acute HCV IV Study was designed as a single arm, prospective multicenter pilot study to evaluate the efficacy and safety of treatment with sofosbuvir plus ledipasvir (SOF/LDV) for 6 weeks without ribavirin in patients with acute genotype 1 HCV monoinfection. We here report the final 24 weeks post treatment results. **Results:** Twenty patients were included by 10 centers (60% male, mean age 46 ± 12 years; 11 patients HCV genotype 1a, 9 patients genotype 1b). The main risk factors for HCV infection were sexual transmission (n = 11) and medical procedures/needle stick injuries (n = 5). Median alanine aminotransferase (ALT) and median bilirubin le-

vels before start of antiviral treatment were 225 U/l (range 32 – 2716) and 13.6 µmol/l (range 5.13 – 111), respectively. ALT levels rapidly declined during therapy and values normalized already by treatment weeks 2 in 9 patients and by week 4 in 17 patients. HCV RNA was undetectable by the Roche COBAS® AmpliPrep/COBAS® TaqMan® HCV Test v2.0 by weeks 2, 4 and 6 in 8, 13, and 20 patients, respectively. SVR-12 was 100% and 19 patients have completed FU-week 24 and all remained HCV-RNA negative. One patient was lost to follow-up at week 24 post treatment. **Conclusion:** Treatment for 6 weeks with LDV/SOF was well tolerated and highly effective in HCV genotype 1 monoinfected patients with acute hepatitis C. Virological response was durable after therapy for at least 24 weeks. A rapid improvement in biochemical disease activity was observed during therapy. Short-duration treatment of acute hepatitis C could prevent the spread of HCV in high risk populations and may be cost-saving as compared to treatment of chronic hepatitis C.

2.38 Specific role of extracellular signal-regulated kinase (ERK) in cold-induced injury to cultured liver endothelial cells

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Background: Hypothermic injury, mediated by an iron-dependent increase in ROS formation that leads to mitochondrial injury, occurs in various cell types and constitutes part of preservation injury. The mitogen activated protein kinases (MAPKs) ERK, JNK and p38 have, among other factors, been implicated in preservation injury. However, their role in cold-induced injury is unclear. Therefore, we here studied activation of ERK, JNK and p38 during/after cold incubation and provide evidence that the ERK phosphorylation is involved in cold-induced injury of rat liver endothelial cells. **Methods:** Rat liver endothelial cells of two cell lines were incubated at 4 ° C in Krebs-Henseleit (KH) buffer or UW solution, followed by rewarming in cell culture medium. Cell injury was determined by LDH release. Phosphorylation of MAPKs was assessed by western blot and mitochondrial membrane potential by laser scanning microscopy using the fluorescent indicator TMRM. **Results and Conclusion:** During hypothermia, ERK and JNK were not phosphorylated, in fact, preexisting phosphorylation was significantly decreased compared to control. However, both MAPKs were strongly and transiently phosphorylated during rewarming. In contrast, p38 MAPK showed an opposite pattern of phosphorylation, which was reduced during rewarming. Pretreatment of the cells with an iron chelator (deferoxamine, 10 mM/ml) or Rho-GTPases inhibitor (Toxin-B, 10 ng/ml) partially inhibited ERK phosphorylation. Inhibition of ERK by a MEK inhibitor (U0126, 10 µM/ml), decreased cell injury after cold incubation/rewarming in KH and UW solution (2% vs. 42% and 3% vs. 61% LDH release) respectively, whereas the inactive analogue (U0124, 10 µM/ml) was ineffective. Similar protection was observed with the MEK inhibitor PD 98059. Inhibition of ERK prevented the loss of the mitochondrial membrane potential. In conclusion, the data suggest that iron-dependent ROS and Rho-GTPases contribute to transient activation of ERK after cold incubation which appears to be involved in cold-induced cell and mitochondrial injury.

2.39 The ALBI score: a new tool for decision making after TACE

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Background and aims: Treatment of intermediate HCC with transarterial chemoembolization (TACE) displays a very heterogeneous overall survival (OS). This is largely determined by the degree of underlying liver function. Recently, the ALBI score, a score has been introduced for assessment of liver dysfunction in HCC patients. However, the impact of the ALBI score in the management of HCC patients treated with TACE has not been elucidated previously. Here, we set out to address this important clinical question. **Methods:** 95 patients with newly diagnosed hepatocellular carcinoma who were treated with TACE at our liver center were included in this retrospective analysis. Medical records, laboratory parameters before and 3 months after TACE, response data according to the modified RECIST criteria (v1.1) and OS were assessed. Liver function was determined by using the ALBI score and was correlated with treatment response after TACE and OS. **Results:** The most common cause for HCC was alcoholic liver disease (47.4%) followed by chronic hepatitis C virus infection (21.1%) and non-alcoholic fatty liver disease (12.6%). 68 patients (71.6%) were classified as Child A and 27 patients (28.4%) presented with Child B liver cirrhosis. The majority of patients (78.9%) had intermediate stage HCC (BCLC B). The median ALBI score before TACE was -2.36 [-4.69 – (-0.87)]. Specifically, 34 patients (35.8%) were graded as ALBI 1, 56 patients as ALBI 2 (58.9%) and 5 patients as ALBI 3 (5.3%). In patients with Child A cirrhosis, TACE did not lead to significant changes in the ALBI score. However the ALBI score was able to identify further prognostic subgroups in patients with Child A cirrhosis as ALBI 1 patients displayed a significant better OS compared to patients with ALBI 2 (36 vs. 17 months; $p = 0.022$). In addition, a better ALBI score was also positively associated with the radiological response (= CR, PR and SD) after TACE (-2.49 vs. -1.89; $p < 0.001$). Similar associations were not observed in patients with Child B cirrhosis. **Conclusion:** The ALBI score is able to identify prognostic subgroups in HCC patients with Child A liver cirrhosis and to predict treatment outcome after TACE. Thus, the ALBI score may help clinicians to select patients who may benefit most from TACE in intermediate HCC.

2.40 The Involvement of Tumor Necrosis Factor α in Autoimmune Hepatitis

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Background and Aim: Autoimmune Hepatitis (AIH) is an immune mediated and inflammatory liver disease of unknown etiology. The strong association of the HLA haplotypes DR3 and DR4 indicates that in particular the CD4+ T cell response appears to be crucial in AIH pathogenesis. The aim of this study was to characterize extra- and intrahepatic T cells of AIH patients regarding their gene expression and cytokine production profile. **Methods:** Cytokine expression of circulating peripheral blood mononuclear cells (PBMC) from 8 AIH patients or 10 healthy donors was analyzed in an unbiased approach by gene expression array. Findings were confirmed by qPCR and flow cytometry of PBMC and liver infiltrating lymphocytes (LIL). **Results:** Tumor Necrosis Factor α (TNFα) was highly expressed both in PBMC and LIL in AIH patients, but not of healthy subjects ($p = 0.0021$). Flow cytometry confirmed that the frequency of extrahepatic TNFα+ CD4+ T cells was significantly higher in 15 AIH patients than in 12 healthy controls ($p = 0.0098$). Of note, the LIL of 10 AIH patients manifested significantly increased TNF expression (3.2-fold; $p = 0.036$) compared to the control cohort of 4 patients with adenoma or Nonalcoholic Steatohepatitis (NASH). Moreover, there was a trend towards higher frequencies of intrahepatic TNFα+ CD4+ T cells in a cohort of 6 AIH patients compared to a cohort of 6 control patients with adenoma, NASH or metachronous hepatic metastatic ovarian cancer ($p = 0.0714$). **Conclusion:** TNFα+ CD4+ T cells seem to be overrepresented in PBMC and LIL of AIH patients. These findings might indicate a role of TNFα in the pathogenesis of AIH both in the extra- and intrahepatic T cell-compartment of patients.

2.41 The regulation of inflammasome components in monocytes and macrophages from patients with decompensated cirrhosis

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Background: Inflammation is a major cause of organ failure and mortality in cirrhosis and acute-on-chronic liver failure (ACLF). One major driver of inflammatory damage in a variety of liver diseases is inflammasome complexes, which control the release of pro-inflammatory cytokines and inflammatory cell death. **Aims:** This project aims on investigating regulation of inflammasome activity in cirrhosis and ACLF and its regulation by low-dose genotoxic stress. **Methods:** Circulating monocytes and peritoneal macrophages from patients with decompensation of cirrhosis, and monocytes from healthy controls were isolated and treated with different doses of low-dose epirubicin before the stimulation with inflammasome activators in vitro. **Results:** Monocytes from patients with ACLF expressed the highest levels of the inflammasome

components pro-IL-1β, AIM2 and ASC as compared to monocytes and macrophages from patients without ACLF. Incubation of monocytes with serum from patients with cirrhosis as in contrast to healthy serum increased IL-1β secretion after low-dose LPS and AIM2 activation in vitro. Treatment with low-dose epirubicin prior to inflammasome activation suppressed the inflammasome-mediated IL-1β release in all investigated primary cell types as well in the monocytic cell line THP-1 in a dose-dependent fashion. The down regulation of the inflammasome activation occurred below the threshold of cytotoxicity without quantifiable DNA damage as assessed by gamma-H2AX levels. **Conclusion:** Our data suggest a regulation of the monocyte inflammasome in the context of decompensated cirrhosis and ACLF, which can be counteracted by low-dose genotoxic stress.

2.42 Transiente Elastografie (Fibroscan) zur Risikostratifizierung des hepatorenalen Syndroms (HRS)

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Einleitung: Das hepatorenale Syndrom (HRS) ist – nach Ausschluss anderer zur Einschränkung der Nierenfunktion führenden Erkrankungen – als zunehmende Verschlechterung der Organfunktion bei gleichzeitigem Vorliegen einer akuten und/oder chronischen Leberkrankheit definiert und geht mit einer deutlich erhöhten Mortalität einher. Die genauen Pathomechanismen sind bis heute unvollständig verstanden. Neben peripher arterieller Vasodilatation der Splanchikusgefäße und konsekutiver Vasokonstriktion der Nierengefäße scheinen auch die Stimulation des Sympathikus und die Aktivierung des Renin-Aldosteron-Angiotensin-Systems sowie multiple Zytokine eine Rolle zu spielen. Methoden, welche das Auftreten eines HRS prognostizieren, sind nicht verfügbar. Die vorliegende Arbeit evaluiert in einer monozentrischen retrospektiven Gesamterhebung den Nutzen des Fibroscans zur Risikostratifizierung für die Entwicklung eines HRS. **Methodik:** Zwischen 20.09.2012 und 02.06.2014 wurden am UKS 1.396 Patienten mit Leberzirrhose erfasst, von denen 560 (40,1%) eine transiente Elastografie (Fibroscan-Messung) erhielten und in die weitere Analyse eingeschlossen wurden. Eine Unterscheidung zwischen den verschiedenen Typen des HRS erfolgte nicht. Zur statistischen Analyse wurde ein Cox proportional Hazard Model gewählt. **Ergebnisse und Diskussion:** Die Patienten waren im Mittel 59,9 Jahre alt (56% Männer) und 810 Tage unter Beobachtung; sie stellten sich durchschnittlich 18-mal in der Klinik vor. Die Entwicklung eines HRS wurde bei 65 Patienten (4,7%) dokumentiert, von denen bei 32 lag eine vorherige Fibroscan-Messung vorlag. Es konnte gezeigt werden, das pro kPa Anstieg der Lebersteifigkeit ein 1,8% höheres Risiko besteht, ein HRS zu entwickeln. Hiermit steht erstmalig eine Methode zur Verfügung, die eine Risikostratifizierung von Patienten mit Leberzirrhose hinsichtlich der Entwicklung eines HRS zulässt. Der Ansatz ist prospektiv weiter zu evaluieren.

2.43 Real-world evidence on all-oral, interferon-free regimens with Ombitasvir/Paritaprevir/r and Dasabuvir for treatment of chronic HCV patients receiving opioid substitution therapy in the German Hepatitis C-Registry

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Background: Chronic hepatitis C virus (HCV) infection is highly prevalent in patients with a history of intravenous drug abuse; however historically, implementation of HCV treatment in this patient group in real-world remained challenging. With all-oral, interferon-free HCV regimens available and the favorable setting of opiate substitution treatment (OST), conditions are now potentially more adequate for successful HCV treatment in these patients. In clinical trials with OST patients with chronic HCV genotype 1 (GT1) infection, the regimen of ombitasvir (OBV), paritaprevir (co-dosed with ritonavir [PTV/r]) with dasabuvir (DSV) and/or ribavirin (RBV) achieved a sustained virological response (SVR) rate of 96.4%. However, real-world data on this regimen in this group is currently limited. **Goals:** In this analysis, we report the real-world effectiveness, safety and adherence of the treatment regimen of OBV/PTV/r ± DSV ± RBV in patients receiving OST in the German Hepatitis C-Registry (DHC-R). **Methods:** The DHC-R is a non-interventional, prospective cohort study with more than 300 study sites in Germany. OST patients with HCV GT1 or GT4 infection treated with the regimen of OBV/PTV/r ± DSV ± RBV between February 1, 2014 and December 7, 2015 were analyzed. Effectiveness was assessed by SVR at post-treatment week 12 or 24 (SVR12/24), safety is reported in all patients that received at least one dose of study drug and overall adherence was assessed by quantification of medication taken. **Results:** In total, 67 OST patients received OBV/PTV/r ± DSV ± RBV, with 30 patients having reached post-treatment follow-up. Among patients receiving treatment, 8/67 (12%) had cirrhosis and 31/67 (46%) were treatment-experienced, predominantly to interferon-based therapies. 39/67 (58%), 17/67 (25%) and 11/67 (16%) were infected with HCV GT1a, GT1b or GT4, respectively. All OST patients who reached post-treatment follow-up achieved SVR12/24 (100%; 30/30). Adverse events (AE), predominantly mild or moderate in severity, were reported in 43/67 (64%) of patients. Serious AE were rare (6.0%; 4/67) and treatment discontinuations due to AE were not reported. Overall adherence was very good (100% of medication taken in 27/34; 79%) or good (90 – 100% of medication taken in 6/34; 18%) in the majority of patients. **Conclusions:** In this real-world cohort of HCV GT1 and GT4-infected patients, treatment of patients receiving OST with OBV/PTV/r ± DSV ± RBV was well tolerated and achieved high rates of overall adherence and SVR.

2.44 Real-world evidence on all-oral, interferon-free regimens with Ombitasvir/Paritaprevir/r and Dasabuvir for treatment of chronic HCV patients with renal insufficiency in the German Hepatitis C-Registry

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Background: Chronic hepatitis C virus (HCV) infection is common in patients with renal insufficiency and end-stage renal disease, but clinical data on HCV therapy in this patient group is limited. The direct-acting antiviral regimen of ombitasvir (OBV), paritaprevir (co-dosed with ritonavir, PTV/r) with dasabuvir (DSV) with or without ribavirin (RBV) was approved in 2015 in Germany for treatment of patients with chronic hepatitis C genotype 1 (GT1) and 4 (GT4) infection. However, real-world data on this regimen in patients with renal insufficiency and end-stage renal disease is currently limited. **Goals:** In this analysis, we report the real-world effectiveness and safety of OBV/PTV/r ± DSV ± RBV in patients with renal insufficiency in the German Hepatitis C-Registry (DHC-R). **Methods:** The DHC-R is a non-interventional, prospective cohort study with more than 300 study sites in Germany. Patients with an eGFR < 90 ml/min/1.73 m2 and chronic HCV GT1 or GT4 infection treated with OBV/PTV/r ± DSV ± RBV between February 1, 2014 and December 7, 2015 were analyzed. Effectiveness was assessed by SVR at post-treatment week 12 or 24 (SVR12/24) and safety is reported in all patients that received at least one dose of study drug. **Results:** In total, 326 patients with renal insufficiency received OBV/PTV/r ± DSV ± RBV. 52/326 (16%) patients had moderate (eGFR 30 – 60 ml/min/1.73 m2; n = 36) to severe (eGFR < 30 ml/min/1.73 m2; n = 16) renal insufficiency (incl. 8 patients

on hemodialysis). 85/326 (26%) had cirrhosis and 202/326 (62%) were treatment-experienced. 61 (19%), 229 (70%), 7 (2%) and 29 (9%) were infected with HCV GT1a, GT1b, an unspecified GT1-subtype or GT4, respectively. Of 193 patients who reached post-treatment follow-up, 96% (152/159) of patients with mild renal insufficiency and all patients (100%; 34/34) with moderate to severe renal insufficiency achieved SVR12/24. Adverse events (AE) were reported in 188/326 (58%) of patients, specifically in 109/160 (68%) who received RBV and in 79/166 (48%) who did not. The rate of treatment discontinuations due to AE was 6.3% (10/160) in patients treated with RBV and 0.6% (1/166) in patients treated without RBV. Clinically significant changes in renal function were not observed when comparing eGFR at baseline and end of treatment in the overall population (Δ eGFR: – 2.1 (mean)± 10.8 (SD) ml/min/1.73 m2) or in different subgroups, incl. patients with severe renal insufficiency. **Conclusions:** In this real-world cohort of HCV GT1- and GT4-infected patients, treatment of patients with renal insufficiency with OBV/PTV/r ± DSV ± RBV was well tolerated and achieved high rates of SVR, irrespective of baseline renal function.

3. Metabolism/Transport

3.1 Cholestasis is associated with induction of antimicrobial active reduced human β-defensin 1

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Background & Aims: Despite a multitude of microbial challenges, knowledge about innate antimicrobial defense of the liver is limited. We systematically investigated expression and regulation of hepatic antibacterial peptides and post-translational modification of human beta defensin-1 (hBD-1). **Methods:** Different defensins including hBD-1 and its activating factor thioredoxin-1 (TXN) were analyzed in healthy and cholestatic human liver samples by qPCR and immunostaining. Regulation of hBD-1 expression was studied in different in vitro cell systems and in vivo using bile duct ligated mice (BDL mice). Regulation pathways of bilirubin and bile acids were studied using siRNA-mediated knockdown of potential nuclear receptors. **Results:** We found major expression of hBD-1 and TXN – a known activator of hBD-1 antimicrobial activity – in hepatic cells whereas other β-defensins were minimally expressed. Using a specific antibody for the reduced active form of hBD-1 we found strong active peptide expression in co-localization with TXN in human hepatocytes. hBD-1 was upregulated in cholestasis using two independent clinical cohorts. In cholestatic mice hepatic AMP expression (mBD-1 and Hamp) was also enhanced. Bilirubin and bile acids were able to induce hBD-1 in hepatic cell cultures in vitro. Treatment with siRNA and/or agonists demonstrated that the farnesoid X receptor (FXR) mediates basal expression of hBD-1, whereas both CAR and FXR seem to be responsible for the induction of hBD-1 by bilirubin. **Conclusions:** hBD-1 is the major defensin in the liver and is further induced during cholestasis through bilirubin and bile acids, mediated by CAR and especially FXR. Reduction of the peptide by TXN post-translationally modifies and activates hBD-1.

3.2 Vitamin D3 improves liver histology and hepatic gene expression in a murine obesity/NASH model independently of intestinal Fgf15 induction

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Introduction: The gut hormone FGF19 (FGF15 in mice) induces various metabolic effects that could help to treat obesity and NAFLD. Previous studies demonstrated that Fgf15 transcription is induced by short-term VD3 administration. In this study, we analysed whether long-term VD3 treatment ameliorates NASH in an obese mouse model and whether this is associated with changes in intestinal Fgf15 expression. **Methods:** To induce obesity and NASH, C57BL/6J mice were fed a high-fat/high-sugar diet (HFSD) with low VD3 for 16 weeks. The effects of preventive (starting at wk1) and interventional (wk12) VD3 treatment were studied on the level of liver histology and hepatic/intestinal gene expression. **Results:** Animals receiving HFSD with low VD3 developed obesity, histologically-defined NASH and liver fibrosis after 16 weeks. This phenotype was associated with increased expression of lipogenic, inflammatory and pro-fibrotic genes in the liver and with decreased Fgf15 levels in the intestine. Interestingly, preventive but not interventional VD3 treatment resulted in improvements of liver histology, including a significant decrease of steatosis and a trend towards a lower NAFLD activity score. In line with these histological changes, preventive VD3 treatment improved gene expression in the liver. However, these effects occurred in the absence of increased intestinal Fgf15. **Conclusions:** This study reveals a beneficial impact of VD3 treatment on disease progression in a murine obesity/NASH model. Importantly, our observations suggest that timely initiation of VD3 supplementation (preventive vs. interventional) is critical for treatment outcome. In the applied NASH model, VD3 seems to act independently of the bile acid-regulating hormone FGF15.

3.3 Generation of a 3D model to better mimic NAFLD in vitro

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Introduction: Non-alcoholic fatty liver disease (NAFLD) has become one of the major risks for the development of hepatocellular carcinoma (HCC). Simple steatosis, which is the first stage of NAFLD, is characterized by abnormal lipid accumulation in hepatocytes. As the molecular processes which lead to steatosis and further progression to non-alcoholic steatohepatitis (NASH), fibrosis, cirrhosis and HCC, are currently not completely understood, many in vivo and in vitro models have been established. However, current existing in vitro models are wrought with limitations. Liver-biopsy derived primary hepatocytes have several limitations: They (i) are rare with a low number of healthy donors, (ii) have high inter-donor variability, (iii) show limited expansion in culture and (iv) rapid decline in function. Thus, the generation of hepatocyte like cells (HLCs) from induced pluripotent stem cells (iPSCs) can provide an alternative cell source. So far these cells lack full maturity even though they express ALBUMIN and cytochrome P450 family members. Mature HLCs are needed to maximize the relevance of the experimental outcome and applicability of these cells for toxicology and drug screening. Improved maturity and functionality of human iPSC-derived HLCs has been achieved employing three-dimensional (3D) approaches incorporating MSCs and endothelial cells. **Methods:** Our preliminary proof of principle experiments involved mixing of iPSC-derived mesenchymal stem cells (iMSCs) with human umbilical vein endothelial cells (HUVECs) and HepG2 cells to generate 3D in vitro liver organoids. Furthermore it is planned to generate MSCs, HLCs and endothelial cells from the same iPSC line (same genetic background). Additionally spinner flasks were used to provide better medium flow and to improve liver bud growth. These liver buds were then challenged with high levels of glucose and insulin to mimic steatosis. **Results:** Within three weeks these cells aggregated and formed vascularized liver buds when cultured on artificial extracellular matrices. These buds secrete urea, express ALBUMIN, VIMENTIN (MSC marker) and CD31 – an endothelial specific marker. Fat droplet formation after challenging with glucose and insulin resulted in activated expression of steatosis-associated genes. **Discussion/Conclusion:** These iPSC-derived liver organoids have the added advantage of having present mesenchymal and endothelial cells from the same individual. Further studies are underway to better characterize these liver buds both molecular and biochemically for liver associated genes, pathways and functions. This 3D iPSC-based approach is a good model to study steatosis and complements our current iPSC-based 2D model.

3.4 Chronic Renal Failure Is Associated With the Development of NAFLD/NASH

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Background: Chronic renal failure (CRF) is frequently associated bone metabolism and osteoporosis with lowered levels of Vitamine D and/or hyperparathyreodism particular in case of hemodialysis. Younger studies suggest an association of low vitamin D levels with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), as well as metabolic syndrome, and diabetes mellitus. Unfortunately, a causality could not yet be proven. Our aim was to identify patients on higher risk to develop NAFLD/NASH in a selected patient cohort being admitted for renal disorders. **Material and Methods:** 176 patients, admitted to the department of nephrology of the University Hospital Marburg for renal disorders whose plasma vitamin D concentration, phosphate and parathormone levels and liver enzyme levels had been quantified beforehand, were enrolled and a retrospective investigation of laboratory parameters (including electrolytes, hormones, and vitamins) and pre-existing medical conditions (including high blood pressure, diabetes, hyperlipoproteinaemia, and more) followed. Appropriat statistical test were used to characterise the cohort (ANOVA; MANN-Whitney-U; FISHER-EXACT) using SPSS™. Other hepatopathies were excluded. Steatosis was assessed by ultrasonography. **Results:** Patients were divided into 4 groups according to plasma vitamin D levels (normal > 25 ng/ml; low < 25 ng/ml) and transaminase levels (AST/ALT/γ-GT > 30 U/l; normal: AST/ALT/γ-GT < 30 U/l). Low 1,25-hydroxyvitamin D levels correlated signifactly with high kreatinine, urea, and LDL levels, while low 25-hydroxyvitamin D levels correlated with high cholesterol and trigylceride levels, suggesting a relationship between low vitamin D levels and fat metabolism disorders. Interestingly end stage renal failure (chronic hemodialysis) was significantly correlated with the development of NAFLD/NASH with significantly higher levels of AST/ALT and gGT, hyperparathyreodism and hyperphosphatemia Transaminases were significantly lower if Vitamin D was supplemented **Discussion:** Vitamin D deficiency is often present in patients with kidney diseases such as chronic renal failure. Vitamin D levels are correlated to age and sex of the patient. Patients suffering from renal failure are on high risk developing NAFLD/NASH if diminished vitamin D levels are present. Supplement of Vitamin D saves from NAFLD/NASH. The correlation of hyperparathyreodism and NAFLD/NASH has to be further investigated in larger patient groups.

3.5 Association of Elevated Liver Enzymes and Vitamin D Deficiency

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Background: Vitamin D deficiency is a frequent burden in northern countries, mainly caused by decreased intake and reduced sun exposure. Clinical manifestations of vitamin D deficiency are often skeletal, like osteoporosis, osteomalacia, and rickets. In addition, low vitamin D levels are associated with chronic renal disease, muscle weakness, hypertension, cardiovascular events, and diabetes. Younger studies suggest an association of low vitamin D levels with non-alcoholic fatty liver disease (NAFLD) and non-alcoholic steatohepatitis (NASH), as well as metabolic syndrome, and diabetes mellitus. Unfortunately, a causality could not yet be proven. Our aim was to assess the role of vitamin D levels in relationship with elevated liver enyzmes and life-style diseases such as hypertension, diabetes, and fat metabolism disorders. **Material and Methods:** 181 patients, whose plasma vitamin D concentration and liver enzyme levels had been quantified beforehand, were recruited and a retrospective investigation of laboratory parameters (including electrolytes, hormones, and vitamins) and pre-existing medical conditions (including high blood pressure, diabetes, hyperlipoproteinaemia, and more) followed. **Results:** Patients were divided into 4 groups according to plasma vitamin D levels (normal > 25 ng/ml; low < 25 ng/ml) and transaminase levels (AST/ALT/γ-GT > 30 U/l; normal: AST/ALT/γ-GT < 30 U/l). A significant correlation of low vitamin d levels and elevated liver enzymes could not be shown. Nevertheless, low 1,25-hydroxyvitamin D levels correlated with high kreatinine, urea, and LDL levels, while low 25-hydroxyvitamin D levels correlated with high cholesterol and trigylceride levels, suggesting a relationship between low vitamin D levels and fat metabolism disorders. **Discussion:** Our results suggest that

- The prevalence of vitamin D deficiency directly correlates with the season (winter>summer).
- Vitamin D deficiency is often present in patients with kidney diseases such as chronic renal failure.
- Vitamin D levels are correlated to age and sex of the patient.
- A significant correlation of vitamin D deficiency and elevated liver enzymes can only be shown if a very low cut-off is chosen.
- Vitamin D deficiency is associated with metabolic syndrome.

3.6 HBV infection alters the transcriptional regulation of FXR-dependent microRNAs

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The binding of HBV to the Na+-taurocholate cotransporting polypeptide (NTCP) was previously shown to limit its function, leading to reduced nuclear translocation of the bile acid sensor farnesoid X receptor (FXR) and alterations in the expression of key genes of hepatic cholesterol and bile acid metabolism in human hepatocytes in vivo (Oehler, Hepatology 2014). Furthermore, we showed that the capacity of FXR agonists to modulate bile acid homeostatic genes was strongly weakened by HBV infection (Bhadra, HBV international meeting, 2015). MicroRNAs play an important role in cellular processes and possibly in HBV infection. Moreover, growing evidence indicates that FXR is involved in transcriptional regulation of hepatic miRNAs. Aim of this study was to investigate (I) the impact of FXR activation mediated by FXR-agonists on miRNAs expression in primary human hepatocytes in vivo and (II) to assess whether HBV can interfere with FXR-mediated miRNA induction. **Methods:** Humanized uPA/SCID/beige mice with stable HBV infection treated with FXR-agonist PX20606 (0.2 mg/day, 3 week-oral feeding), while uninfected and vehicle fed infected mice served as controls. MicroRNA expression was determined by using a novel TaqMan™ Advanced miRNA Assay which includes miRNA amplification and reverse transcription after RNA phenol/chloroform purification. MicroRNA in situ hybridization was performed with the Affymetrix® viewRNA chemistry. **Results:** Treatment with PX20606 caused a significant induction of miR-144 in the uninfected animals (2.42- fold change relative to untreated, uninfected controls; p = 0.01). Notably, no enhancement of miR144 was observed in HBV infected mice receiving the FXR-agonist. A similar behavior could be observed for miR-451, which is expressed in a cluster with miR-144. In contrast to previous reports based on HCC analyses, PX20606 administration did not induce miR-122 in uninfected animals, suggesting differential miRNA regulation in tumor and primary hepatocytes. Flourescence in situ hybridization of miR-144 has approved these findings and also revealed dissimilar expression patterns in murine and human hepatocytes. Both miR-144 and miR-451 are highly abundant in the serum of chimeric mice. **Conclusion:** Chronic HBV infection hinders FXR-dependent cellular pathways, thus altering the transcription not only of genes involved in bile acid and cholesterol metabolism, but also of FXR-targeted hepatic microRNAs.

3.7 A cholesterol-containing modified western diet inducing steatohepatitis (NASH) with insulin resistance in wildtype B6 mice

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Obesity is associated with insulin resistance and type II diabetes but also with non-alcoholic fatty liver disease (NAFLD) and steatohepatitis (NASH) that are hepatic manifestations of the metabolic syndrome. One of the most relevant factors contributing to obesity is diet. Feeding studies with rodents using diets like high-fat-, methionine-choline-defi-

cient- or paigen-diet often did not induce the same phenotype like in human metabolic syndrome. Here we designed a new high-fat-containing western-diet that caused obesity, insulin resistance and NASH in mice. C57BL/6-mice fed a western-diet with cholesterol (WD-C, 25 g/100 g fat) or high-fat-diet (HFD, 25 g/100 g fat) for 20 weeks significantly gained weight, increased their body fat mass and were glucose intolerant and slightly hyperinsulinaemic compared to chow-fed mice. Serum parameters for liver inflammation were elevated after feeding a WD-C but not after feeding a western-diet (WD, 25 g/100 g fat) or HFD. Histological scoring of the liver revealed steatohepatitis with fibrosis (NASH) in WD-C-fed mice and only simple steatosis without inflammation in WD- and HFD-fed mice. Gene expression analysis detected an up-regulation of chemokines, pro-inflammatory cytokines, immune cell infiltration and induction of markers for fibrosis and apoptosis only in livers of WD-C-fed mice. Serum level of triglycerides and cholesterol were slightly elevated, but liver triglycerides and cholesterol were highly increased in WD-C-fed mice compared to chow-fed mice. Cholesterol induced expression of chemotactic and inflammatory cytokines in cultured Kupffer cells and rendered cultured hepatocytes more susceptible to TNFα/actinomycinD-induced apoptosis. Mice fed a WD-C therefore are a potential better model for human metabolic syndrome and NASH than mice fed a HFD.

3.8 Activation of inflammation and cholestasis by Egr-1 is linked to liver regeneration by regulating ALR expression

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Introduction: cholestasis is defined as the impairment of bile flow in the liver leading to bile acids accumulation within the liver, promoting inflammation and therefore causing liver injury. Previous studies indicated the role of bile acids in the up-regulation of early growth response fator-1 (Egr-1) which initiates the inflammation through up-regulating several pro-inflammatory genes. The hepatotrophic factor Augmenter of liver regeneration (ALR), with anti-oxidative and anti-apoptotic properties, was demonstrated to support liver regeneration after hepatic injury. Nevertheless, little is known about the impact of ALR in cholestasis. Therefore, the aim of our study was to investigate the potential role of key bile acids and Egr-1 in regulating ALR expression, and to determine a potential role of Egr-1 in the regeneration process mediated by ALR. **Results:** Analysis of ALR promoter showed three potential response elements for Egr 1. Reporter-gene assays, using two different constructs of ALR promoter demonstrated increased promoter activity upon treatment with various bile acids or co transfection with Egr-1 in hepatoma cells compared to control. Nevertheless, co transfection with dn-Egr-1 diminished the induced promoter activity. Furthermore, we found induced levels of ALR protein in bile acid-treated HepG2 cells and Egr-1-overexpressing HepG2 cells compared to control. We further performed EMSA technique and demonstrated the specific binding of Egr 1 to its response element within ALR promoter in liver cells. **Conclusion:** Our data indicate that bile acids-induced Egr-1 regulates ALR as a marker of liver regeneration in cholestasis.

3.9 Bone morphogenetic protein (BMP)-9 enhances high glucose-mediated microvascular damage

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Bone morphogenetic protein (BMP)-9, a member of the TGF-β superfamily of cytokines is produced in the liver. Culturing hepatic stellate cells (HSC) under conditions of high glucose was reported to enhance HSC activation in vitro. Using real-time PCR we now found that such culture-conditions also lead to significantly enhanced expression of BMP-9. Since BMP-9 is a secreted cytokine, circulating with the blood stream this observation led us to investigate possible consequences of glucose-mediated elevation of BMP-9 levels on endothelial cells. In macrovascular endothelial cells (HUVEC) as well as liver sinusoidal endothelial cells (LSEC) and primary mouse hepatocytes, BMP-9 stimulation induced an upregulation of VEGF expression as determined by real-time PCR. In LSEC as well as brain microvascular endothelial cells BMP-9 induced expression of capillarization markers like fibronectin and Integrins αv and β3. In line with this observation, we found by immunohistochemistry a deposition of fibronectin within the sinusoids of diabetic mouse livers. These data suggest that systemic elevations of BMP-9 levels occur under hyperglycaemic conditions and that BMP-9 aggravates high-glucose-mediated microvascular damage. These findings further imply that application of BMP-9 neutralizing agents might be a promising new strategy to protect patients from diabetes-induced microvascular damage in the future.

3.10 Combined accumulation of hepatitis B surface antigen and mutated alpha-1 antitrypsin promotes the development of liver disease via autophagy overload and activation of p62-mTOR-Nrf2 axis

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Background & aim: Liver constitutes a major protein secreting organ and because of that, accumulation of secretory proteins in the endoplasmatic reticulum (ER) constitutes a hallmark of multiple liver disorders. Among them, aggregation of mutated α1-antitrypsin termed as PiZ is a characteristic feature of AAT deficiency, whereas retention of hepatitis B surface protein (HBs) is commonly found in chronic hepatitis B infection and gives rise to ground glass hepatocytes. To study the effect of these proteotoxic situations, we crossbred PiZ and HBs overexpressing animals. **Aims & methodology:** Mouse and human livers with combined PiZ/HBs retention were evaluated by quantitative RT-PCR, immunoblotting, histological/immunological staining and biochemical assays. DHE staining was employed to visualize oxidative stress and p62-containing aggregates were isolated via MACS. Crossbreeding of PiZ mice with CHOP knockouts was performed to address the role of CHOP in proteotoxic injury. **Results:** HBs-PiZ mice were viable and developed normally. At 2 months of age, they displayed a significantly stronger liver injury (ALT: PiZ-HBs 88, HBs 53, PiZ 32, $p < 0.05$ for both). While both AAT and HBs were retained in the ER, both in human and the mice, the inclusions displayed a distinct, non-overlapping pattern. Among 10 months old animals, PiZ-HBs mice displayed a more pronounced dysplastic changes, liver fibrosis and higher hepatocyte proliferation. At 14 months of age, double transgenic animals developed larger tumor nodules (Double 141, HBs 36, PiZ 3.7 mm2, $p < 0.05$ for both) and a higher tumor load. Analysis of 16 gene expression signature revealed a more aggressive tumor subtype in HBs-PiZ mice as evidenced by down-regulation of Cyp2e1, Aqp9, Apoc4, ($p < 0.001$ for all) and C1 s ($p < 0.05$). As the presumable drivers of liver phenotype, HBs-PiZ animals exhibited a marked accumulation of the autophagy adaptor protein p62 that was observed in the protein overloaded hepatocytes. Moreover, an activation of p62-mTOR axis with increased pmTORS2448 and p4EBP1 levels was detected. p62 MACS uncovered a precipitation of otherwise soluble HBs and accumulation of ubiquitinated proteins in the double-transgenic animals, but not in HBs mice. As a result of increased proteotoxic stress, an accumulation of reactive oxygen species and an activation of Nrf2 signalling was detected in HBs-PiZ animals. NF-kB activation along with strong CHOP overexpression evidenced an activation of ER overload response. However, this response does not seem to contribute to the observed phenotype, since the ablation of CHOP did not alter the extent of liver injury and fibrosis in 19 months old PiZ/PiZ-CHOP-KO mice. **Conclusions:** Our results suggest that a combined accumulation of PiZ and HBs accelerates the development of liver injury due to autophagy overload with subsequent activation of p62-mTOR-Nrf2 axis.

3.11 Differential role of NLRP3 inflammasome during acute and chronic cholestatic liver injury

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Cholestasis causes hepatic toxicity which leads to liver injury by cell death. Apoptotic and pyroptotic cell death is a consequence of caspase-8 and/or caspase-1 activation and is mediated by the NLRP3 inflammasome. NLRP3 activation triggers liver inflammation, fibrosis and hepatocyte pyroptosis in mice. Thus we here studied the role of NLRP3 inflammasome activation during cholestatic liver injury. To investigate the role of the NLRP3 inflammasome we performed bile duct ligation (BDL) in WT and NLRP3 -/- mice. We were interested to study the role of NLRP3 during acute (first 48 hours) and chronic (28 days) injury after BDL. Inflammation, fibrosis and cell death were evaluated with qPCR, IHC, IF and western blot analysis. Acute cholestatic liver injury in NLRP3 -/- mice resulted in a significantly stronger increase in serum transaminases compared to WT mice. In addition hepatic inflammation, as shown by mRNA levels of TNFα, IL-1b, MCP-1 and influx of Ly6G + Cd11b + cells by FACS analysis was observed. The increase in liver injury can be explained by a stronger necroptotic response in NLRP3 -/- mice, as shown by an increase in mitochondrial ROS and RIPK1 and 3 expression. In contrast, during chronic cholestatic liver injury, lack of NLRP3 expression was protective as evidenced by reduced serum transaminases, less inflammation and reduced fibrosis. TUNEL and caspase-3 staining additionally showed decreased cell death in NLRP3 -/- mice. Furthermore, caspase-3 staining showed clusters of increased cell death in WT mice 28 d after BDL, characteristic for pyroptosis. This can be explained by the inability of NLRP3 -/- mice to activate caspase-1 and release IL-1b. Due to the increased RIP3-mediated necroptosis in NLRP3 -/- mice expansion of progenitor/oval cells is triggered which could explain reduced liver injury. In conclusion, during acute cholestatic liver injury lack of NLRP3 expression is associated with increased liver injury mediated by mitochondrial ROS and RIPK1 and 3 activation triggering increased necroptosis and a stronger inflammatory response. In contrast, in the chronic phase NLRP3 -/- mice have less liver injury due to reduced pyroptotic cell death leading to less inflammation and fibrosis.

3.12 Differentiated Liver Damage by Acetaminophen in a Human Ex-Vivo Liver Model

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Introduction: Reliable test systems to identify hepatotoxicity are essential to study mechanisms of liver damage of known drugs and to predict unexpected drug-related liver injury of new drug candidates. **Methods:** A human-based ex-vivo liver model was established, which was able to keep hepatic functionality for up to 30 hours. Drug-induced liver injury was investigated using acetaminophen (APAP) as model substance. Hourly samples from the perfusate were taken for measurement of general metabolism and clinical parameters. Liver function was assessed by clearance of indocyanine green (ICG) at 4, 20, and 28 hours. MiR-122 was measured in perfusate collected during time periods 8 – 19 h (outflow1) and 19 – 30 h (outflow2). APAP was applied after 8 hours of perfusion with 6.5 mg per gram of perfused liver tissue. **Results and Conclusions:** Six pieces of untreated human liver specimen maintained stable liver function over the entire perfusion period indicated by ICG half-lives of 14.8 ± 0.4 (4 h), 15.4 ± 1.4 (20 h) and 19.1 ± 2.9 min (28 h). Out of seven liver sections incubated with low-dose acetaminophen, three revealed strong damage with ICG half-lives significantly higher than in non-treated livers after 20 and 28 hours of perfusion ($p < 0.005$). Four liver sections revealed weak damage with significantly higher ICG half-lives only after 28 hours of perfusion ($p < 0.05$). The different extent of liver damage was confirmed by significant higher levels of miR-122 in outflow 1 and 2 ($p = 0.02$) found only in experiments with strong damage. The presented model allows investigation of hepatotoxicity in human liver tissue upon applying drug concentrations relevant in humans. The system reflects well the inter-individual differences upon drug response observed in patients.

3.13 Elevated release of extracellular vesicles in obese patients with no-alcoholic fatty liver disease (NAFLD)

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Introduction: Obesity is excessive fat accumulation in adipose tissue and its prevalence has increased dramatically worldwide. Non-alcoholic liver disease (NAFLD) represents the liver manifestation of the metabolic syndrome and is closely associated to obesity. Prevalence rates of NAFLD increase in parallel to the obesity pandemic. Extracellular vesicles (EV) are recently identified membrane-encased vesicles that are secreted from cells. Our study aimed to analyze the effect of bariatric surgery on liver-related EV release and to identify markers contained in liver-specific EV. **Methods:** Sera were collected from severely obese patients with NAFLD ($n = 23$) before and after bariatric surgery and form normal weight subjects ($n = 12$). EV isolation was performed from frozen serum samples by ultracentrifugation followed by PEG precipitation and EV quantification using NTA system. **Results:** The number of EV in obese patients was significantly higher than in normal weight subjects ($p < 0.0001$). Over a period of 6 weeks after bariatric surgery a slight increase in EV numbers was observed (n. s.). Total protein content of EV increased after bariatric surgery compared to before surgery. Furthermore, the number of EV and BMI were positively correlated. **Conclusion:** Increased EV release occurred in obese patients with NAFLD. Bariatric surgery led to slight increases of EV numbers and protein content, possibly suggesting a change of EV composition. In depth analysis of biological background and characterization of EV by surface proteins and content is warranted.

3.14 Establishment of Copper Resistance in an ATP7B Knockout Cell Line

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Copper (Cu) is an essential trace element to all living organisms. However, intracellular Cu levels have to be strictly regulated to avoid toxicity. The effects of a disturbed Cu homeostasis become obvious in Wilson's disease (WD), a genetic disorder caused by mutations in the ATP7B gene. In addition, Cu transport pathways also play an important role in other diseases (e.g. Alzheimer's disease). Studying the mechanisms by which Cu resistance may be acquired will therefore not only give further insights on how cells control Cu levels, but possibly also result in new therapeutic strategies for several diseases. Studying the mechanisms by which Cu resistance may be acquired will therefore not only give further insights on how cells control Cu levels, but possibly also result in new therapeutic strategies for several diseases. HepG2 ATP7B Knockout cells were cultivated in medium containing 0.1 mM Cu and cell growth was monitored by trypan blue stainings. Prolonged exposure to Cu (> 55 days) led to a 17-fold higher survival rate in HepG2 ATP7B Knockout cells as compared to untreated control HepG2 ATP7B Knockout cells when exposed to a concentration of 1 mM Cu, as analyzed by MTT assay (34.3 ± 4.6% vs. 2.4 ± 0.8%). However, termination of Cu administration (weaning) did not re-establish Cu sensitivity in these Cu resistant (CuR) cells. RT-qPCR analysis revealed altered expression patterns for various genes involved in Cu homeostasis in CuR in comparison to untreated cells. Multiple changes in gene expression profiles were transient (e.g. upregulation of MT1 and downregulation of CTR1), whereas others were persistent and did not depend on continuous Cu administration. As likely candidates to support Cu resistance (e.g. CTR2, OCT3, OCTN2) those genes were further investigated. Cu accumulation was determined by atomic absorption spectrometry. Protein expression was assessed by flow cytometry and Western Blot analysis. Here we show for the first time a Cu resistant hepatoma cell line that lacks the important Cu transporter ATP7B. The acquired Cu resistance seems to be stable as determined by

weaning experiments. Our cellular model may give further insights into the pathophysiology of WD and the mechanisms of Cu homeostasis.

3.15 Protein quality control system in hepatocyte-like cells derived from induced pluripotent stem cells obtained from FAP patients

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Familial amyloid polyneuropathy (FAP) is caused by mutations in the transthyretin (TTR) gene which leads to severe neurological impairments. Recently, several genes of the protein quality control system (PQC) were identified in association to FAP. These studies mostly relied on investigations of whole liver or affected tissues. Since TTR is expressed and synthesized by hepatocytes, analysis of gene expression profiles in hepatocytes is needed. Hepatocyte-like cells (HLCs) were differentiated from induced pluripotent stem cells (iPSCs) which were generated by transfection of urine-derived cells from FAP patients carrying different TTR variants with pluripotency factors Sox2, Oct3/4, Klf4 and c-myc. The hepatic character of HLCs was assessed by functional analysis, gene expression profiling and immunostainings. RT-qPCR was used to analyze the gene expression related to PQC. The TTR protein expression was determined by Western Blot. HLCs derived from FAP patients showed high expression of hepatic markers, like albumin and transferrin. TTR mRNA expression in HLCs was almost identical to primary human hepatocytes. 42 genes related to PQC were analyzed and seven genes were significantly affected (fold change >±5) in at least two patients as compared to healthy HLCs. Six out of seven genes are associated with the extracellular co-chaperoning. In addition, some of these genes were specifically altered when HLCs of different genotypes were compared. Furthermore, two genes of the unfolded protein response were significantly altered (fold change >±5) when asymptomatic were compared to symptomatic patients. Our data suggest that HLCs derived from FAP patients are an excellent model to study patient-specific disease mechanisms, including the role of the protein quality control system.

3.16 Genetic and biochemical screening for Gaucher disease and lysosomal acid lipase deficiency in patients with hepatosplenomegaly of unknown etiology

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Background: Gaucher disease and lysosomal acid lipase deficiency (LALD) are rare lysosomal lipid storage diseases caused by mutations of the GBA and LIPA genes, respectively. These mutations cause deficiency of the lysosomal enzymes β-glucocerebrosidase and acid lipase. Both diseases result in hepato- and/or splenomegaly and an increase of the serum surrogate marker chitotriosidase, which represents a macrophage activation marker. **Patients and methods:** In this study we screened patients with hepatosplenomegaly of unknown origin at a tertiary academic referral center. We determined the enzymatic activities of chitotriosidase, β-glucocerebrosidase and acid lipase with biochemical arrays. Common LIPA and GBA gene variants were genotyped with Taqman assays and direct sequencing. **Results:** Between 2010 and 2014 we identified 201 patients with hepato- and/or splenomegaly of unknown etiology origin at our center (median age 55 yrs [21 – 83]; 69 women [34%]). Among them, 136 patients (67.7%) presented with spleen size > 110 mm; hepatomegaly was diagnosed in 100 patients (49.8%), and combined hepatosplenomegaly was present in 35 patients (17.4%). Overall, we identified 36 patients (27.4%) with serum chitotriosidase activities ≥ 250 nmol/ml/h. β-glucocerebrosidase and acid lipase activities were within normal ranges in all patients tested. In this cohort, we could identify a single heterozygous carrier of the mutation p.N370S in the GBA gene only; the mutation p.L444P was not detected. The E8SJ mutation in the LIPA gene was not observed in our patients either. Chitotriosidase activities declined significantly with age (-2,66 nmol/ml/h, p < 0,003), and lysosomal acid lipase showed a seminar trend. **Discussion:** Apparently hepatologists do not miss a large number of patients with lipid storage disease. For the identification of unknown patients with Gaucher disease or LALD, screening efforts should not only include liver centers but other units specialized in pediatric care, lipid disorders and orthopedics. In addition, newborn screening for Gaucher disease and LALD might be considered.

3.17 Gut Microbiota as a Target in T cell- mediated hepatic Injury

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Introduction: The microbiota contributes to various auto-immune and liver diseases. It remains unclear whether the immunological response is modulated by the microbiota directly, or indirectly by its metabolites like short chain fatty acids (SCFAs) or bile salts. These are important signaling molecules in gut and liver. Currently, little is known about the influence of microbiota in immune-mediated liver injury. We investigated the role of gut microbiota in the model of Concanavalin A (ConA), which is a well-established mouse model of T-cell mediated hepatitis. **Methods:** Germ free C57BL/6 or antibiotic-treated FIRxtiger mice were challenged with ConA and analyzed during initial, peak and recovery phase of the disease. The influence of SCFAs on immune-cell composition was analyzed via flow-cytometry after in vitro co-culture experiments w/o liver sinusoidal endothelial cells (LSECs). **Results:** Germ free and antibiotic-treated mice were protected from ConA-induced hepatitis. Antibiotic-treatment reduces CD4+T-cells and IL-10+ regulatory T-cells (Tregs) frequencies following ConA. Hepatic expression of the butyrate receptor GPR-43 and CYP7A1, an enzyme in the bile acid pathway, were up-regulated in response to antibiotic-treatment and down-regulated after ConA-challenge. The opposite effects were shown for the bile acid membrane receptor TGR5. In vitro butyrate reduces interferon-γ and IL-10 production by CD4+T-cells in co-culture with LSECs. **Conclusion:** ConA-damage was ameliorated by reduction or absence of the gut microbiota. Reduced IL-10+Tregs in the liver are in line with less severe hepatitis. Our results indicate that further analysis of SCFAs and bile salt metabolism is needed to determine its specific function during pathogenesis of liver disease.

3.18 Hemolysis-mediated suppression of hepcidin causes hepatic iron overload in alcoholic liver disease

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Background: Patients with alcoholic liver disease (ALD) develop prognostically unfavourable hepatic iron overload (HIO) and anemia, however, the underlying molecular mechanisms and the role of the systemic iron masterswitch hepcidin are poorly understood. Herein, we investigate the effect of hemolysis in the disruption of the physiological hepcidin regulation. **Methods:** Iron-related parameters, hepcidin (mRNA and serum levels), molecular and laboratory markers were studied in a large cohort of Caucasian heavy drinkers (n = 831, age range 22 – 87 years, mean alcohol consumption 192 g/day). Hemolysis was further studied in age-matched C57BL/6 mice injected (i. p.) for 2 consecutive days with 60 mg/Kg body weight of phenylhydrazine (PHZ). Histology, iron stain, mRNA and protein expression were measured both in liver and spleen. Erythrophagocytosis was finally studied in differentiated primary human macrophages fed with oxidized red blood cells (RBCs). **Results:** Indirect evidence for hemolysis (anemia, high ferritin, high LDH, high MCV) as cause for HIO was found in 16.4% of a large population of heavy drinkers. Notably, further indicators of hemolysis (haptoglobin, B12, folic acid) were not significantly changed most likely due to impaired liver synthesis. Despite higher ferritin levels as compared to controls, hepcidin was not adequately upregulated in the hemolysis group suggesting a suppressive effect of hemolysis. We next recapitulated suppression of hepcidin in a murine model of PHZ-induced hemolysis. PHZ induced severe anemia, elevated transaminases, transferrin saturation and LDH within 24 hours. In the same time period, heme oxygenase 1 was highly induced while hepcidin was significantly lowered by 50%. Histology confirmed an increased number of phagocytosed RBCs in the spleen and iron-loaded Kupffer cells in the liver. We finally studied in vitro the process of erythrophagocytosis in primary human macrophages exposed to oxidized RBCs. Notably, hepcidin showed a biphasic response. At physiological low

levels of oxidized RBCs, hepcidin was induced while it was strongly suppressed at higher levels of hemolysis. **Conclusion:** Our data suggest that masked hemolysis and suppression of hepcidin seems to be an important mechanism of hepatic iron overload in patients with ALD.

3.19 Independent and additive induction of hepcidin by hypoxia and H2O2: Evidence for NOX4-mediated iron signaling

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Background: Hepcidin is the systemic masterswitch of iron homeostasis that plays an important role in the accumulation of carcinogenic hepatic iron in most chronic liver diseases. Iron, inflammation and H2O2 are all potent inducers of hepcidin but the role of additional hypoxia is poorly understood. We here study the hepcidin response under hypoxia and H2O2 simulating an environment typical found in chronic liver disease. **Material and Methods:** Both, hypoxia chamber and the newly introduced enzymatic GOX/CAT (catalase/glucose oxidase) system were used to independently control low steady state (ss) H2O2 levels and to maintain low oxygen level (1 and 5% O2) in Huh7 hepatoma cells. Cells were preconditioned to different oxygen levels (1, 5 and 21% O2) for 24 h and then co-exposed to low ss H2O2 over 24 h. Furthermore, the involvement of NADPH oxidases (NOXs), especially NOX4, the major NOX found in liver was studied and pharmacologically inhibited by VAS2870. Hepcidin and NOX4 mRNA levels were studied by qRT-PCR. Cellular oxygen and H2O2 levels were assessed by HIF1alpha or Prx2 western blot. **Results:** Hypoxia (1 and 5% O2) generated either by hypoxia chamber, the GOX/CAT system or the hypoxia-mimetic CoCl2 caused a robust 1.5 – 2 fold increase of hepcidin mRNA levels during 24 h. Hypoxia-mediated induction of hepcidin was further potentiated during co-exposure of cells with low ss H2O2 levels. In fact, hypoxia and H2O2 acted independently and in an additive manner on hepcidin mRNA. Both, Prx2 expression and HIF1alpha stabilization mirrored intracellular H2O2 and changes in oxygen levels. Notably, NOX4, the major liver-expressed NOX was also strongly upregulated during hypoxia, suggesting a mechanistic role in mediating hepcidin upregulation. Pharmacological inhibition of NOXs by VAS2870 led to decreased hepcidin mRNA levels back to baseline levels found in the controls. **Conclusions:** We here demonstrate for the first time that hypoxia alone is able to induce hepcidin independent of and in addition to low ss H2O2. Our mechanistic studies point towards the oxygen-consuming and H2O2-generating oxidase NOX4 as potent inducer of hepcidin ultimately causing carcinogenic liver iron accumulation.

3.20 Hepatitis B virus production is enhanced through early autophagy, but degraded through autophagosome-lysosome fusion

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Background and aims: Autophagy is a catabolic process that mediates the removal of long-lived proteins and damaged organelles through a lysosomal degradative pathway. Recently, several studies reported that celluar autophagy may have complex interconnections with hepatitis B virus (HBV) replication and envelopment. However, the definite mechanisms of different stages of the autophagic process affecting HBV production are unclear. **Methods:** In the present study, we investigated whether and how HBV production was modulated through early and late autophagy. Two proteins Rab5 and -7 related to the formation of early autophagic bodies and autophagosomes were chosen to study their roles in HBV replication. **Results:** HBV replication, HBsAg secretion, and intracellular HBsAg expression were significantly decreased by Rab5 silencing. The intracellular level of HBsAg was strongly decreased about 40% by Rab5 silencing. These results were consistent with previous findings that blocking initiation of autophagy by silencing the components of ULK1 complex or using 3-MA significantly reduced HBV replication and HBsAg formation. In constrast, silencing Rab7 significantly enhanced HBV replication and HBsAg production. The level of intracellular HBsAg increased about 33% by Rab7 silencing. Interestingly, Rab7 silencing led to the promotion of autophagic flux without reduced autophagic protein degradation. In reverse, Rab7 overexpression significantly decreased the level of HBV replication and HBsAg production. Considering the function of Rab7 in autophagosome formation, our results suggest that the delivery of HBV capsids and HBsAg to the lysosome may facilitate their degradation. Thus, enhanced HBV replication and HBsAg production by Rab7 silencing was a result of blocking the fusion of autophogosome with lysosome. This hypothesis could be confirmed by silencing or overexpression of the tethering genes SNAP29 and VAMP8, which are involved in mediating autophagosome-lysosome fusion, showing the same effect on HBV replication of HBsAg. In the same line, the blockage of lysosome function by chloroquine increased HBV replication and HBsAg formation. Confocal microscopic analysis directly demonstrated the accumulation of HBsAg in autophagosomes in the presence of chloroquine. **Conclusion:** Our findings provide new evidence indicating that HBV replication and HBsAg production requires early autophagic peocess but will be degraded to a significant part in autophagosome compartment.

3.21 Hepatocyte KLF6 regulates autophagy in a p53 dependent manner

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Autophagy regulates turnover of long-lived or damaged organelles and proteins, which promotes survival and regeneration by maintaining intracellular energy production. In acute liver injury, autophagy helps to reduce cellular stress and tissue damage in hepatocytes. KLF6 is a transcription factor and tumor suppressor gene belonging to the family of zinc finger proteins that regulate potential target genes and mechanisms by binding to specific DNA motifs. In patients and several models of acute liver injury, we observed a consistent induction of hepatic KLF6 expression that was accompanied by an induction of autophagy. Here, we aimed to investigate a potential interaction of KLF6 and autophagy related molecules by transfecting HepG2 cells with a specific KLF6 expressing vector. In this model, KLF6 overexpression led to a significant induction of autophagy as assessed by LC3-II Western blot. In order to elucidate a potential interaction of KLF6 with the autophagy related molecules Atg7 and Beclin1, we performed co-transfection studies with specific Atg7 or Beclin1 luciferase reporter plasmids in KLF6 overexpressing cells. KLF6 overexpression led to a significant transcriptional activation of Atg7. In HepG2 cells, we did not observe a significant induction of Beclin1. Using chromatin immunoprecipitation (ChIP) we could show that KLF6 actively binds to the promoter region of Atg7. Since p53 is an important mediator in autophagy induction, we used p53-deficient HepG2 – 303 cells and repeated luciferase reporter experiments. Interestingly, in KLF6 overexpressing HepG2 – 303 cells, Atg7 luciferase activity was not altered, while Beclin1 luciferase activity was significantly induced in KLF6 overexpressing p53 knockout cells. These experiments demonstrate, that KLF6 activates autophagy by binding directly to Atg7 promoter region or Beclin1 and therefore influencing autophagy induction in a p53 dependent manner.

3.22 Hsp72 overexpression protects from liver injury and hepatocellular death via attenuation of oxidative stress and JNK-signaling

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Background & aims: Heat shock protein (Hsp) 72 is a molecular chaperone that is upregulated in response to a variety of stress situations and possesses broad cytoprotective functions. To determine its hepatic function, we studied its expression in various human liver disorders and its biological significance in newly generated transgenic animals. **Aim & methods:** Transgenic mice overexpressing Hsp72 under the control of a tissue-specific tetracycline-inducible system were crossed with animals

carrying the tetracycline-responsive transactivator under the control of the liver activator protein promoter (Hsp72-LAP mice). Acute liver injury was induced by a single intraperitoneal injection of acetaminophen (800 mg/kg). 8 week feeding with methionine choline-deficient (MCD) and 12 week exposure to 0.1% 3,5-diethoxycarbonyl-1,4-dihydrocollidine-supplemented diet (DDC) was used to induce lipotoxic liver damage and Mallory-Denk body (MDB) formation, respectively. Primary hepatocytes were subjected to treatment with palmitic acid (0,5mM, 24 h). **Results:** Patients with non-alcoholic steatohepatitis and chronic hepatitis C infection displayed elevated Hsp72 levels. Hsp72 levels progressively increased with the extent of hepatic inflammation. Hsp72-LAP mice exhibited doxycycline-regulated, robust Hsp72 overexpression in hepatocytes, but not in the other tissues or cell types. Primary hepatocytes isolated from these animals were more resistant towards the isolation-induced stress. In all liver injury models (DDC, MCD, APAP), Hsp72-LAP mice displayed lower ALT levels. Hsp72 overexpressors also had a lower amount of APAP protein adducts ($p = 0.03$) and showed protection towards oxidative stress and APAP-/MCD-induced cell death. In all tested models (isolated hepatocytes, APAP/MCD-treated mice), Hsp72-LAP mice/hepatocytes displayed significantly attenuated JNK activation. On the other hand, overexpression of Hsp72 did affect the extent of steatosis neither after MCD treatment nor after palmitic acid exposure. **Conclusions:** Our results demonstrate that Hsp72 overexpression exhibits a broad hepatoprotective function via attenuation of oxidative stress, hepatocellular death and JNK signaling.

3.23 Impact of morphogenic pathways on liver metabolism

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Background: Morphogens like Hedgehog (Hh) and Wnt/β-Catenin are known to govern developmental processes in embryogenesis, tissue differentiation and tissue regeneration. More recently the impact of those morphogens on adult cell physiology and metabolism has sparked increasing interest. Metabolic pathways in the liver are strongly zonated, partially caused by adapting to changing contents of nutrients and oxygen within the porto-central axis. How Morphogens contribute to the lobular distribution of metabolic pathways is part of an evolving field in science. Long time pericentral located key player Wnt and its periportal located zonation-keeper APC were thought to be exclusively responsible for liver zonation. Recently our group could demonstrate that inhibition of the Hh-pathway leads to increased liver lipid accumulation which resembles a picture of non-alcoholic liver disease. As it is known for Wnt/β-Catenin, Hh signaling likely influences other metabolic activities. For a better understanding of metabolic liver zonation under morphogenic control we aimed at demonstrating the mutual impact and zonal distribution of Hh and Wnt signaling. **Methods:** To investigate the mutual impact of Hh- and Wnt-signaling on liver zonation different hepatocyte-specific transgenic mice models were breeded. These mice, allowing investigating the mutual impact of Hh and Wnt/β-Catenin signaling by activation or inhibition of Wnt and Hh. Hh activation was aimed by Ptch1 deletion, leading to loss of Smo repression. Hh inhibition was achieved by Smo knockout. Wnt/β-Catenin signaling was enhanced by targeting the zonation keeper APC. To depict the porto-central distribution of different pathway markers liver slices were stained by immunohistochemistry and in situ hybridization. **Results:** Our results indicate a crosstalk between Wnt and Hh in the liver parenchyma. Furthermore we could also show that upregulation of Wnt signalling is associated with a pericentral expansion of Indian Hh. These findings were confirmed using qPCR expression analysis. Our investigation also shows opposite responses for APC complex protein, Axin2, and Wnt receptor Frizzled4 in Hh inactivated and Wnt activated mice. **Conclusion:** Our results indicate an intensive crosstalk between Wnt and Hh. Additional experiments will help to gain greater insight into the mutual influence on porto-central gradient and metabolic zonation of both pathways.

3.24 iPLA2beta deficiency protects mice from diet-induced obesity and steatosis by replenishing the loss of hepatic phospholipids containing unsaturated fatty acids

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It is known that a decrease of hepatic phospholipid (PL) contents is associated with steatosis in non-alcoholic fatty liver disease patients and in rodents. We have previously reported that calcium-independent phospholipase A2 Group VIA (iPLA2beta) deficiency alleviates steatosis in morbidly obese ob/ob KO mice, by replenishing the loss of PL thus supporting the reported role of iPLA2beta in PL remodeling. Here we tested whether iPLA2beta deficiency could protect against diet-induced steatosis and whether hepatic PL composition could be modified. Feeding of WT mice with HFD (35% fat w/w) for 6 months increased body- and liver weights as well as ALT, AST, LDH, ALP, serum lipids (cholesterol and non-esterified fatty acids), and hepatic steatosis scores. All of these parameters were attenuated in whole-body iPLA2beta KO mice fed with HFD. We analyzed PL profiling by LC/MS-MS. Like ob/ob livers where all PL subclasses are decreased, HFD feeding in WT mice did significantly decrease nearly all PL subclasses except for phosphatidylethanolamine (PE). These decreases in phosphatidylcholine (PC), phosphatidylserine (PS) and phosphatidylinositol (PI) could be mainly observed in species containing mono- or polyunsaturated fatty acids (PC 34:1, PC 34:3, PC 34:2, PC 36:3, PC 36:2, PC 36:5, PC 38:5; PS 36:1, PS 36:4; PI 34:2, PI 36:3, PI 36:4, PI 28:4, PI 38:5, PI 40:4), while PE containing saturated C 16:0 and C 18:0 fatty acids PE 34:0 was the only species that increased. This resulted in an increase of the ratio between saturated and unsaturated fatty acids in total PL, PC, and PE. The decrease of total PL, unsaturated PL, and the ratio could be reversed by iPLA2beta deficiency. Consistent with our results in ob/ob livers, iPLA2beta in HFD livers was also specific for the hydrolysis of PC and PE that contained either C 16:0 or C 18:0 fatty acids on sn-1 position. However despite of steatosis protection, three out of six HFD-fed KO mice exhibited ear dermatitis suggesting systemic inflammation likely due to iPLA2beta role in immune cells. Taken together, altered hepatic membrane homeostasis with a loss of especially unsaturated PL occurred during HFD feeding. iPLA2beta deficiency rescued this loss by replenishing unsaturated fatty-acid containing PL hence restoring the membrane balance leading to steatosis protection. This study should be confirmed by using hepatocyte-specific iPLA2-beta KO mice prior to establishing a strategy to inactivate hepatocyte iPLA2beta for treatment of NAFLD.

3.25 L-Selectin (CD62L) drives development and progression of non-alcoholic steatohepatitis (NASH) in two dietary mouse models

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Introduction: Non-alcoholic steatohepatitis (NASH) is the third most common reason for liver transplantation in developing countries and one of the fastest growing medical problems. **Aim:** The significance of infiltrating lymphocytes and how they affect NASH development and progression remains unclear. Therefore, this study investigates the role of the cell adhesion molecule L-Selectin (CD62L) in two different mouse steatohepatitis models. **Methods:** Constitutive L-Selectin-/- mice were fed either MCD diet (methionine and choline deficient) for 4 weeks or HF (high fat)-diet (HFD) for 24 weeks. **Results:** After 4 weeks of MCD and 26 weeks of HFD treatment L-Selectin-/- mice showed a less invasive phenotype in steatosis development and progression compared to WT (wildtype) controls. This attenuated disease pathogenesis was reflected by less histomorphological changes with maintenance of an intact liver architecture and less fatty liver degeneration compared to WT controls. Furthermore, L-Selectin-/- animals displayed a dampened manifestation of typical characteristics of the metabolic syndrome, namely a decreased liver:body weight ratio, an improved glucose tolerance and decreased levels of cholesterol and serum triglycerides in both mouse steatohepatitis models. The amelioration of diet-induced steatohepatitis progression was further reflected by lower levels of serum transaminases and proin-

flammatory cytokines in L-Selectin-/- mice compared to WT. Consistent with the less invasive phenotype, L-Selectin-/- animals showed an increased anti-oxidative stress response by an elevated expression of Nrf2 (Nuclear factor (erythroid-derived 2)-like 2) and HO-1 (Heme oxygenase-1) and an enhanced hepatic immune cell infiltration of TReg cells. Moreover a stronger activation of CD4+ T cells was detected by higher CTLA-4 (cytotoxic T-lymphocyte-associated Protein 4) expression. Those changes finally resulted in a protection of L-Selectin-/- mice from fibrosis progression with less collagen accumulation compared to WT controls. L-Selectin-/- animals showed a significant decrease in Sirius Red stained collagen fibres. **Conclusion:** L-Selectin deficiency leads to a protection of the MCD and the HFD induced steatohepatitis. Therefore, the blockade of the interaction of L-Selectin with its endothelial receptor MAdCAM-1 (mucosal vascular addressin cell adhesion molecule 1) seems to provide a potential novel target for therapeutic interventions during NASH development.

3.26 Modulation der Unfolded Protein Response (UPR) im murinen HBV-Modell durch Aktivierung des Transkriptionsfaktors ATF6

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Einleitung: Ein direkter, intrinsischer Mechanismus der Zellschädigung bei der HBV Infektion beruht auf der Akkumulation der HBV-Hüllproteine (HBs) im Endoplasmatischen Retikulum (ER) der Hepatozyten, was zu einer „Unfolded Protein Response", ER-Stress und zur Apoptose führen kann. Das synthetische Chaperon 4-PBA (Phenylbuttersäure) bewirkt in verschiedenen Modellen für Protein-Aggregationskrankheiten die Auflösung zell- schädigender Aggregate und wird bereits therapeutisch eingesetzt. ATF6 ist ein ER-Stress regulierender Transkriptionsfaktor. Die Akkumulation von falsch gefalteten Proteinen im ER führt zur proteolytischen Spaltung von ATF6. Der cytosolische Teil von ATF6 wird in den Zellkern transportiert und wirkt als Transkriptionsfaktor zur Bildung von Chaperonen. Ziel der vorliegenden Studie ist die Analyse der Wirkung des chemischen Chaperons 4-PBA auf ATF6 im murinen HBV-Modell. **Methoden:** Stabil-HBs exprimierende Zelllinien (HuH7, NIH3T3 und AML12) wurden etabliert und mit PBA behandelt. Die HBs-transgenen Mäuse C57BL/6J-tg(Alb1HBV)44Bri/J wurden über einen Zeitraum von 1 – 8 Wochen kontinuierlich mit PBA (im Trinkwasser) behandelt. Zellkulturexperimente und Tiermodell wurden mittels Immunhistochemie, mRNA-Array, qRT- PCR und Western Blot analysiert. **Ergebnisse:** Die Zugabe von PBA in HBs-transgenen Zelllinien bewirkt eine Feinverteilung bzw. Auflösung der durch Überexpression aggregierten HBV-Hüllproteine. Im HBs-transgenen Mausmodell wird unter PBA-Gabe ein verändertes intrazelluläres Aggregationsmuster mit konsekutiver Feinverteilung des Transgens HBs beobachtet. PBA verursacht zudem eine verstärkte Expression von ATF6 und eine gesteigerte Expression, proteolytische Aktivierung und die nukleäre Translokation von Akut-Phase-Proteinen (Serum Amyloid, Lipocalin, Metallothionein). **Schlussfolgerungen:** Eine Auflösung intrazellulärer Aggregate der HBV-Hüllproteine in vitro und im Tiermodell ist mittels PBA erfolgreich durchführbar. Die Aktivierung von ATF6 induziert eine gesteigerte Expression von Akut-Phase-Proteinen. Daneben könnte diese gezielte Immunmodulation durch Verwendung eines synthetischen Chaperons neue Perspektiven für die HBV-Therapie eröffnen. Die Ergebnisse unserer Studie tragen zum besseren Verständnis der zellulären Pathophysiologie von HBV Oberflächenproteinen bei und zeigen potentielle Wege zur Entwicklung neuer Therapieverfahren auf.

3.27 Protective Role of TGR5 against Portal Hypertension in LCA induced toxic liver damage

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Introduction: TGR5 (Gpbar-1) is a membrane bound G-Protein-coupled bile acid receptor (Kawamata et al. 2003), which is expressed in several different cell types such as hepatic stellate cells (HSCs) and liver sinusoidal endothelial cells (LSECs). Lithocholic acid (LCA) is a hydrophobic secondary bile acid. Mice being fed a diet containing 1% LCA are destined to suffer from a toxic liver damage (Woolbright et al. 2014, Fickert et al. 2006). Liver damage is often accompanied by an elevation of portal pressure. It is assumed that HSCs are able to cause portal hypertension via endothelin-1-induced contraction, which is dependent on their transdifferentiation and intracellular cAMP-levels (Rockey et al. 1996, Reinehr et al. 2001). If TGR5 plays a role during this process has yet to be determined. **Methods:** 8 – 12 week old TGR5 knockout (KO) and wildtype (WT) mice were sacrificed after receiving a diet containing 1% LCA (lithocholic acid) for 4 days. BA species in different body fluids were characterized. Markers for liver damage were measured in serum. Liver histopathology was analyzed by HE-staining and immunohistochemistry. The mRNA expression of different genes in liver was determined by Realtime-PCR analysis. HSC contractile function was tested on collagen matrices. Endothelin-1 expression mRNA and protein level were measured via ELISA and Realtime-PCR analysis, respectively. **Results:** TGR5 KO mice are suffering from a more severe liver damage compared to WT littermates when fed a diet containing LCA as determined by the area of necrosis seen in HE-stainings as well as a higher elevation of serum AST and bilirubin levels. By means of Realtime-PCR-analysis we were able to identify several genes being significantly induced in livers of these mice associated with portal hypertension, e.g. endothelin-1. Invasive measurement of blood pressure revealed the presence of portal hypertension only in LCA-fed TGR5 KO mice. Endothelin-1 induced HSC contraction, which most likely underlies portal hypertension in TGR5 KO, was attenuated after preincubation of WT HSC with a TGR5-Agonist. On top of that, treatment of LSECs, a common source of endothelin-1 in the liver, with a TGR-5 Agonist leads to a significant reduction of endothelin-1 levels. **Conclusion:** In conclusion, TGR5 KO mice suffer from a more severe LCA-induced liver damage. This model helped to unmask a protective role of TGR5 against the increase of portal pressure in toxic liver injury through reduction in endothelin-1 expression and secretion from LSECs as well as attenuated endothelin-1 response of HSCs.

3.28 Role of copper transporter ATP7B in cisplatin resistance in human hepatoma cell lines

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Platinum-containing anticancer agents are widely used in the treatment of cancer. One of the major obstacles in effective treatment is the development of intrinsic or acquired drug resistance to chemotherapeutic agents. Overexpression of copper transporters ATP7A and ATP7B was associated with resistance of many tumors to cisplatin (DDP) treatment. ATP7B is primarily expressed in the liver and its role in hepatocellular carcinoma (HCC) remains unclear. The objective of the present study was to determine the effect of ATP7B expression in DDP resistance in different hepatoma cell lines. ATP7B was overexpressed in four different hepatoma cell lines HepG2, HuH-7, HepRG, and PLC/PRF/5. ATP7B mRNA expression was significantly increased in all ATP7B transfected cell lines. All four ATP7B overexpressing hepatoma cell lines did not show increased resistance to DDP. Drug sensitivity and induction of apoptosis was determined in HepG2 and ATP7B knockout (KO) cells. No significant differences were observed among the two cell lines. Next, DDP resistant cells (HepG2-R and KO-R) were generated by stepwise increase of DDP concentrations in the media. Both cell lines showed increased DDP resistance with normal growth rates compared to parental cell lines. Intracellular DDP accumulation and induction of apoptosis was significantly reduced in HepG2-R and KO-R. Gene expression analysis of copper homeostasis genes and other transporters revealed two candidate genes that were significantly affected in cisplatin resistant cells. Further experiments will explore DDP resistance mechanism by overexpression and downregulation of the candidate genes via plasmid and siRNA transfer. Our results show that native ATP7B and overexpressed ATP7B do not confer DDP resistance in HCC. Exploring mechanistic basis of transporter genes other than ATP7B may help explain resistance to chemotherapeutic drugs in HCC.

3.29 Rolle von Prostaglandin E2 bei der Modulation von aktivierten primären Kupfferzellen der Maus

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Adipositas ist mit Lebererkrankungen wie der nicht-alkoholischen Fettlebererkrankung (NAFLD) assoziiert, die von einer Insulinresistenz begleitet werden kann. Bei der Pathogenese haben Kupfferzellen eine besondere Bedeutung, da sie inflammatorische Mediatoren sezernieren und damit entzündliche Prozesse modulieren können. Einer dieser Mediatoren ist das Fettsäurederivat Prostaglandin E2 (PGE2). Im Hinblick auf eine mögliche Beteiligung an der Entstehung einer hepatischen Insulinresistenz soll die Rolle von PGE2 in der Modulation von aktivierten Kupfferzellen untersucht werden. Primäre Kupfferzellen und Peritonealmakrophagen der Maus wurden durch die Stimulation mit Lipopolysacchariden (LPS) aktiviert, woraufhin die Genexpression von verschiedenen proinflammatorischen Cytokinen (IL-1β, TNFα, IL-6, OSM) und Chemokinen (CCL2, CXCL2) induziert wurde. Die LPS-abhängige Induktion von IL-1β, IL-6, OSM und CXCL2 konnte durch gleichzeitige Stimulation mit PGE2 verstärkt werden, wohingegen jene von TNFα und CCL2 durch PGE2 reprimiert wurde. Weiterhin induziert LPS die PGE2-synthetisierenden Enzyme Cyclooxygenase 2 (COX2) und mikrosomale PGE Synthase 1 (mPGES1). LPS-abhängig gebildetes PGE2 könnte im Folgenden über eine autokrine Rückkopplungsschleife über die PGE2-Rezeptoren EP2 und EP4 die Genexpression in Kupfferzellen modulieren. Die PGE2-Rezeptoren wurden dafür in Kupfferzellen mit entsprechenden Antagonisten gehemmt und exemplarisch die Genexpression von TNFα untersucht. Die PGE2-vermittelte Hemmung der LPS-induzierten TNFα-Bildung wurde durch die kombinatorische Stimulation mit den Antagonisten aufgehoben. In Kupfferzellen aus mPGES1 defizienten Mäusen wurde die LPS-abhängige Induktion von TNFα durch die Antagonisten hingegen nicht verringert, was auf eine reduzierte LPS-abhängige PGE2- Bildung durch die mPGES1-Defizienz zurückzuführen ist. PGE2 könnte somit durch die Modulation der Expression von Cytokinen in Kupfferzellen inflammatorische Prozesse in der Leber beeinflussen.

3.30 The liver regeneration associated factor ALR attenuates IL-6 induced acute-phase reactants during hepatic inflammation

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Background and aims: Inflammatory cytokines such as Interleukin-6 (IL-6) play a key role in triggering acute phase response (APR) in the liver upon injury. In response to hepatic injury, the hepatic protein synthesis shifts from hepatic constitutive to acute phase proteins (APPs) including fibrinogen-β (FGB), haptoglobin (HP), C reactive protein (CRP) and serum amyloid A (SAA). Augmenter of Liver Regeneration (ALR), a hepatotrophic factor supporting liver regeneration, was reported to be upregulated after liver damage. The aim of our study was to investigate the expression and synthesis of acute phase proteins in liver cells upon stimulation with ALR. **Methods:** HepG2 cells and primary human hepatocytes were treated with IL-6 (25 ng/ml) with or without ALR (100 ng/ml) for 6, 12 and 24 hours. The expression of HP, FGB and SAA was measured by RT-PCR and western blot techniques and the APP levels in the supernatant were determined by ELISA. **Results:** We found that ALR attenuated the IL-6 dependent increase of HP mRNA levels and reduced HP protein levels in the supernatant. In addition, we could demonstrate that ALR reduces mRNA and protein expression of both FGB and SAA in liver cells. **Conclusion:** Our data show that IL-6 stimulated APP expression was attenuated in the presence of ALR. We identified ALR as a novel regulator of the hepatic APP genes during inflammation. Our findings suggest that ALR could modify cytokine signaling leading to mitigate the acute phase response, a crucial event during liver regeneration. Keywords: Acute phase response, ALR, liver regeneration, inflammation

3.31 The membrane-bound bile acid receptor (TGR5) controls the immune response against Listeria monocytogenes

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Introduction: TGR5 is a G-protein-coupled bile acid receptor found in many cell types of the liver including Kupffer cells. Activation of TGR5 in macrophages or Kupffer cells by bile acids inhibits production of proinflammatory cytokines, chemokines and attenuates the development of atherosclerosis (Kawamata et al., 2003; Keitel et al., 2008; Wang et al., 2011; Pols et al., 2011). Aim of the present study was to determine the anti-inflammatory properties of TGR5 in an animal model for pathogen defense. **Methods:** Male, 8 – 12 week old TGR5 knockout (KO) and wildtype (WT) mice were injected intravenously (i.v.) with 8 × 104 CFU/ml Listeria monocytogenes (L.m.) and observed for 7 days. At different time points (1 – 4 d) after infection with 2.5 × 104 CFU/ml L.m. bacterial titers were determined in spleen and liver. Serum levels of AST and ALT were analyzed using Spotchem-biochemical analyzer. Cytokine levels were analyzed in serum using Luminex cytometric bead assay. Flow cytometry has been used to examine immune cells in non-lymphoid tissues. mRNA expression in liver and spleen was quantified by realtime PCR in relation to an endogenous control (HPRT1 or SDHA). Serum bile acids were analyzed by UHPLC-MS/MS. **Results:** Following L. monocytogenes (L.m.) infection TGR5 KO displayed a 50% mortality rate within a week of inoculation, while 90% of the TGR5 WT animals survived the infection. In addition, three days after infection higher L.m. titers were detected in liver and spleen of TGR5 KO as compared to WT mice. Moreover, the levels of AST and ALT were significant higher in serum from TGR5 KO animals as compared to the WT littermates. Flow cytometry staining revealed an increase in inflammatory cells in livers from TGR5 KO mice. Furthermore, TGR5 KO mice had increased cytokine levels after L.m. infection as compared to WT littermates. TGR5 mRNA expression was significantly up-regulated in WT mice at day 3 and 4 after L.m. infection. Serum bile acid composition was more hydrophobic and thus more cytotoxic h in TGR5 KO following L.m. infection as compared to their WT littermates. **Conclusion:** TGR5 KO mice were more susceptible to L.m. infection as demonstrated by increased mortality rates and elevated serum transaminases. Furthermore, TGR5 KO mice showed, raised Listeria titers, increased liver inflammation, elevated serum inflammatory cytokines and a more hydrophobic serum bile acid composition after L.m. infection. The TGR5 mRNA expression was significantly up-regulated in WT mice following L.m. infection, indicating that TGR5 may confer protective effects in the wildtype animals.

3.32 Xenobiotika-abhängige Regulation der Dejodase 1 in Hepatozyten

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Xenobiotika können mit dem Schilddrüsenhormonsystem interferieren, sodass in der Folge der Energieumsatz gesenkt wird. So führte die wechselseitige Induktion der nukleären Rezeptoren Arylhydrocarbon-Rezeptor (AhR) und Constitutiver Androstan Rezeptor (CAR) zu einer Induktion von UDP-Glucuronosyltransferasen (UGTs) und resultierte in einer erhöhten Glucuronidierung und damit Inaktivierung von Schilddrüsenhormonen in Rattenhepatozyten. Weitere wichtige Enzyme des Schilddrüsenhormonstoffwechsels sind Dejodasen. Es sollte untersucht werden, ob auch die Dejodase 1, die unter anderem T4 zum inaktiven rT3 umwandelt, Xenobiotika-abhängig und CAR- oder AhR-vermittelt reguliert wird. Die Aktivität des Ratten-Dejodase 1-Promotors wurde durch AhR-Liganden in primären Rattenhepatozyten gesteigert. Eine potentielle AhR-Bindestelle im Promotorbereich des Ratten-Dejodase 1-Gens konnte in silico identifiziert werden. Benzo[a]pyren (BaP) induzierte AhR-abhängig die Dejodase 1-mRNA-Expression, erhöhte aber nicht die hepatische Dejodase 1-Enzymaktivität. CAR-Aktivatoren steigerten die mRNA-Expression der Dejodase 1 nicht, verstärkten jedoch den induzierenden Effekt des AhR-Agonisten BaP. Dies ist auf die CAR-abhängige Induktion des AhR zurückzuführen. Obwohl die mRNA-Expression nicht durch den CAR-Aktivator Phenobarbital (PB) reguliert wurde, erhöhte PB die Dejodase 1-Enzymaktivität. Neben den UGTs wurden auch Sulfotransferasen Xenobiotika-abhängig induziert. Die Expression der Sulfotransferase 1b1, die in Ratten T4 sulfatiert, wurde durch PB, BaP und die Kombination aus beiden Substanzen signifikant gesteigert. Dies begünstigt die Dejodie-

rung des inneren Rings von T4 durch die Dejodase 1 und damit die Umwandlung von T4 zu rT3. Xenobiotika könnten demnach auch über eine CAR- oder AhR-vermittelte Induktion der Expression und Aktivität der Dejodase 1 die Dejodierung von T4(-Sulfat) am inneren Ring erhöhen und durch die beschleunigte Inaktivierung von T4 zu rT3 in den Metabolismus der Schilddrüsenhormone und somit in die Steuerung des Energiestoffwechsels eingreifen.

4. Tumors/Liver Surgery

4.1 Annexin A10 meets the challenge of differentiating intrahepatic cholangiocarcinoma from metastatic pancreatic ductal adenocarcinoma. A comparative study of immunohistochemical markers

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Aims: Discriminating intrahepatic cholangiocarcinoma (ICC) from hepatic metastases of pancreatic ductal adenocarcinoma (mPDAC) can be challenging. While pathologists still depend on clinicians' anamnestic readings about primary tumor, their diagnosis will lead the patient either to possibly curative operation (for ICC) or to palliation (for mPDAC). Beyond the validation of recently published potential biomarkers for PDAC (primary or metastatic) in a large cohort, we assessed the diagnostic performance of the most promising candidates in the challenging task of discriminating metastatic PDAC (mPDAC) and ICC. **Methods:** In a training set of 87 ICC and 88 pPDAC, our by proteomic approach previously identified biomarkers Annexin A1 (ANXA1), ANXA10 and ANXA13 were tested in a comprehensive comparison to 11 published biomarkers or -panels (MUCIN 1, Agrin, S100P, MUC5 AC, Laminin, VHL, CK 17, N-Cadherin, ELAC2, PODXL and HSPG2). The biomarkers with best results were applied to an independent clinical condition set each including biopsies of 27 ICC and 36 mPDAC. **Results:** Five markers with the highest AUC values (between 0.72 and 0.84) for the discrimination of ICC and pPDAC, namely Annexin A1, Annexin A10, MUC5 AC, CK17 and N-Cadherin, were applied to liver biopsies containing ICC or mPDAC. Diagnostic characteristics were evaluated for individual markers as well as for 3x panels. ANXA 10 showed the highest diagnostic potential (75.0% and 85.2% correctly classified mPDAC (sensitivity) and ICC (specificity) respectively) of all single markers. **Conclusion:** Our results suggest that ANXA10 may be useful meeting the challenging task of differentiating ICC from mPDAC based only upon specimen.

4.2 Hepatic expression of oncogenes Bmi1 and Dkk1 is up-regulated in hepatitis B virus surface antigen-transgenic mice in vivo and can be induced by treatment with HBV particles or lipopolysaccharides in vitro

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Previous studies have shown that hepatocellular carcinoma (HCC) develops more frequently in hepatitis B virus surface antigen (HBsAg)-transgenic mice (Alb/HBs) than in wild-type (WT) mice. However, the mechanism of this HCC model has not been well documented. Toll-like receptor 4 (TLR4) signaling probably links innate immunity and HCC progression. The current study was designed to investigate the role of innate immunity in hepatocarcinogenesis in Alb/HBs mice. Immunohistochemical analysis of liver specimens from Alb/HBs mice (16 per group) showed that the oncogenes Bmi1 (16/16, 100%) and Dkk1 (13/16, 81.25%) were highly expressed in Alb/HBs mice, whereas the other oncogenes evaluated were expressed in smaller percentages of mice (Afp, 9/16, 56.2%; Ctnnb1, 5/16, 31.3%; Epcam, 0/16; 0%). Comparable results were obtained by quantitative PCR analysis. Interestingly, hepatic gene expression of Tlr4 and TNF was additionally elevated in Alb/HBs mice. In vitro stimulation of primary murine hepatocytes and Hepa1 – 6 cells with cell culture-derived HBV particles or LPS increased the expression of Bmi1 and Dkk1. Proliferation and colony formation of hepatoma cells (Hepa1 – 6) were enhanced by treatment with HBV and LPS and were impaired by the suppression of Bmi1 and Dkk1 by small interfering RNAs. Moreover, substantial induction of BMI1 and DKK1 was found in liver biopsy samples from patients with HBV-related HCC but not in samples from HCC patients without HBV infection. These findings suggest that TLR4 may link inflammation and tumor progression during chronic HBV infection and may promote the progression of HBV-related HCC through BMI1 and DKK1.

4.3 Incomplete RFA causes cell cycle Arrest and enhanced tumor growth – in vitro and in vivo

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Background: Radiofrequency ablation (RFA) is a widely used palliative treatment option of small solid liver tumors. After RFA 3 areas can be histologically distinguished: total thermal necrosis, a hemorrhagic margin and a rim of sublethal damaged cells going into apoptosis. If RFA is incomplete patients seem to suffer from enhanced tumor growth and spread. The aim was to identify the clinical observable mechanism in vitro and in vivo. HepG2 HCC cells were treated with RFA for 10 s, 20 s and 30 s in vitro. Cells were cultured for further experiments at standard conditions. Cells were tested for apoptosis by DIOc6 and PI/Anexin staining. Chamber slides were stained for TUNEL reaction. Cell damage was assessd by luciferase assay for release of adenylate kinase into the supernatant. Activity of caspases 3/7 and 8 were assessed by luciferase assay. Nfkb activation was visualized by EMSA. Bcl-2, BAX, and ER-stress markers BiP and CHOP were measured by RT-PCR and western blotting Cell cycle was assessed by PI staining and flow cytometry. 20 NMRI mice were injected with 5 Mio. HepG2 cells s.c. and were randomized into 2 groups. Group 1 was left without further ttreatment while group 2 was treated with RFA with a power intake of 5 Ws into the tumor center. 30 days after treatment mice were autopsied and examined for tumor size, TUNEL and proliferation by PCNA **Results:** In vitro 30 s RFA led to immediate necrosis. 20 s caused apoptosis and aponecrosis 4 h after treatment. 10 s RFA caused in 9.7% cells apoptosis. Sublethal damaged cells showed S phase arrest. Bcl-2 was significantly upregulated while pro apoptotic bax was significantly suppressed in vitro and in vivo as well. Nfkb activation was only seen in cases treated with RFA. Animals treated with 5 Ws RFA showed even an enhanced tumor progression compared to animals left without treament. The Chaperon BiP was significantly upregulated after RFA in vitro while proapoptotic ER-stress related CHOP was not elevated after sublethal RFA in vitro and in vivo. Ther was no significant difference of BiP in vivo in both groups. **Discussion:** Incomplete RFA causes S phase arrest and enhanced tumor growth by antiapoptotic bcl-2. Bcl-2 and ER-stress could be therapeutic target for future multimodal aadjuvant therapies for local tumor ablation.

4.4 Activation of tumor initiating cells during anti-angiogenic therapies promotes tumor progression and relapse formation in hepatocellular carcinoma

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Introduction: Neo-angiogenesis is frequently observed during progression of hepatocellular carcinoma (HCC) and often associated with poor clinical outcome. However, development of chemoresistance and relapse

formation is observed in the majority of patients. Compelling evidence suggest that tumor-initiating cells (TICs) may contribute to the acquisition of resistant properties, 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 formation of relapse in HCC during anti-angiogenic therapies and define the concomitant adaptive molecular changes. **Methods:** Several HCC cell lines and primary HCC isolates were continuously exposed to sorafenib and sunitinib for 2 weeks. Treatment effects on TICs were estimated by sphere forming capacity in vitro as well as the side-population (SP) approach. Expression levels of key oncogenic and TIC markers were assessed by qRT-PCR and flow cytometry. Furthermore, whole transcriptome analyses were performed at different time points. **Results:** Both treatment regimens effectively reduced oncogenic properties in all investigated HCC cells. However, sustained anti-proliferative effects were observed in only two cell lines whereas an initial treatment effect was subsequently followed by rapid re-growth in the majority of HCC cells thereby mimicking the responses observed in patients. While anti-oncogenic effects in sensitive cell lines were associated with significant reduction sphere forming capacity, TIC markers as well as SP cells, resistant cell line showed a transient increased in TIC properties. Importantly, acquired resistance to both drugs uniformly developed in the cell lines suggesting that common molecular mechanisms might be operative. These adaptive molecular changes involved signaling pathways known to be associated to cell survival (ERK, AKT, MYC), proliferation (TP53, CDKN1A) as well as angiogenesis (VEGF, PDGFR, HIF1a). Furthermore, the resistant cell lines showed compensatory upregulation of key oncogenic molecules such as EGFR as well as multidrug resistance ABC transporters. **Conclusion:** Our in vitro model recapitulates features of drug resistance observed in human HCC patients. Resistance to anti-angiogenic therapies might be fueled by transient expansion of TICs. Therefore, specific targeting of TICs as well as pro-oncogenic compensatory signaling pathways might be an effective therapeutic strategy to overcome resistance in HCC.

4.5 Analysis of molecular mechanisms of 5-fluorouracil-induced hepatic steatosis and inflammation in vitro and in mice

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Chemotherapy-associated steatohepatitis is attracting increasing attention because it heralds an increased risk of morbidity and mortality in patients undergoing surgery because of liver metastases. The aim of this study was to develop in vitro and in vivo models to analyze the pathogenesis of 5-fluorouracil (5-FU)-induced steatohepatitis. **Methods:** Primary human hepatocytes and HepG2 hepatoma cells were incubated with 5-FU at non-toxic concentrations up to 24 h. Furthermore, hepatic tissue of C57BL/6N mice was analyzed 24 h after application of a single 5-FU dose (200 mg/kg body weight). **Results:** Incubation with 5-FU induced a significant increase of hepatocellular triglyceride levels. This was paralleled by an impairment of mitochondrial function and a dose- and time-dependently increased expression of fatty acid acyl-CoA oxidase 1 (ACOX1), which catalyzes the initial step for peroxisomal β-oxidation. The latter is known to generate reactive oxygen species, and consequently, expression of the antioxidant enzyme heme oxygenase 1 (HMOX1) was significantly upregulated in 5-FU-treated cells, indicative for oxidative stress. Furthermore, 5-FU significantly induced c-Jun N-terminal kinase (JNK) activation and the expression of pro-inflammatory genes IL-8 and ICAM-1. Also in vivo, 5-FU significantly induced hepatic ACOX1 and HMOX1 expression as well as JNK-activation, pro-inflammatory gene expression and immune cell infiltration. **Conclusion:** We identified molecular mechanisms by which 5-FU induces hepatocellular lipid accumulation and inflammation. Our newly developed in vitro and in vivo models can be used to gain further insight into the pathogenesis of 5-FU-induced steatohepatitis and to develop therapeutic strategies to inhibit its development and progression.

4.6 Xanthohumol, a prenylated chalcone derived from hops (Humulus lupulus), inhibits hepatic metastasis

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In many cancer entities, including melanoma, hepatic metastasis is the critical factor determining tumor associated mortality. Xanthohumol, a prenylated chalcone derived from hope cones, is known to possess a broad spectrum of chemopreventive and anticancer activities. However, its functional effect on melanoma cells had not been analyzed and no in vivo studies of Xanthohumol effects on metastasis of any tumor entity existed. The aim of this study was to analyze the effect of Xanthohumol on (hepatic) metastasis of melanoma cells. **Methods:** Functional effects of Xanthohumol on proliferation and migration of human melanoma cell lines MelJu, MelIm and SKMel12 were analyzed in vitro. Furthermore, Xanthohumol effects on hepatic metastasis were analyzed in a syngeneic murine model (splenic injection of murine b16 melanoma cells in C57/BL6 mice). Xanthohumol was applied via subcutanously implanted pellets (Innovative Research of America, Sarasota, USA), releasing Xanthohumol continuously over time with a daily dose of 10 mg/kg body weight. Control mice received control pellets. In addition to macroscopic and histological analysis of the livers, expression of MIA, a gene specifically expressed in melanoma cells, was analyzed to measure hepatic metastasis. **Results:** Initially, we analyzed the effect of Xanthohumol on human melanoma cell lines in vitro. Here, Xanthohumol exhibited dose-dependent cytotoxic effects on melanoma cells beginning in the dose-range of 10 – 20 µM. Notably, incubation with more than 10-fold higher Xanthohumol concentrations did not affect the viability of primary human hepatocytes in vitro. Functional analysis revealed that incubation with Xanthohumol in the subtoxic range dose-dependently inhibited proliferation and migration of melanoma cells in vitro. In the in vivo model, liver weight and hepatic MIA expression were significantly lower in Xanthohumol treated mice. Furthermore, size of tumors formed in the liver of Xanthohumol treated mice was smaller and they revealed significantly larger areas of central necrosis compared to control mice. **Summary and Conclusion:** Xanthohumol inhibits tumorigenicity of melanoma cells in vitro and significantly reduced hepatic metastasis of melanoma cells in mice. Furthermore, the observed missing toxicity of Xanthohumol in primary human hepatocytes confirmed previous studies, which demonstrated the safety of even long term application of daily Xanthohumol doses as high as 1000 mg/kg body weight. Together, these data indicate Xanthohumol as a promising agent for treatment of hepatic (melanoma) metastases.

4.7 C-Reactive Protein the Best Survival Predictor in 100 HCCs with Portal Vein Thrombosis after Radioembolization with Yttrium-90

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Background: Sorafenib is the standard line of care which is recommended for advanced hepatocellular carcinoma (HCC). Selective internal radiotherapy (SIRT) with Yttrium-90 microspheres is a novel transarterial approach for patients with advanced HCC. HCC with portal vein thrombosis in the absence of extrahepatic disease are considered in our center as local advanced HCC, who are offered radioembolization. The aim of this study was to validate evidence on safety, long term efficacy and survival probabilities with subgroup analysis in HCC associated with portal vein thrombosis (PVT). **Patients & Methods:** During the time period between 2012 and 2015, one hundred patients had unresectable advanced hepatocellular carcinoma with portal vein thrombosis had been treated with radioembolization using Yttrium-90 glass microspheres. Treatment was performed in a lobar fashion through the right or left hepatic artery. Evaluation of different clinical and laboratory parameter that could predict better survival outcome before treatment with

radioembolization with Yttrium-90 microspheres. Also Post-treatment evaluation and grading of AEs according to CTC v3. **Results:** Among 100 patients had malignant PVT hepatocellular carcinomas, were treated in 142 sessions (mean: 1.6 sessions/patient). The baseline liver disease at time of presentation was Child-Pugh Class A and B in 70% and 30% respectively. Portal vein thrombosis was presented in all cohort classified into 52% branched PVT and 48% main PVT. The median overall survival was 6.1 months (95% CI: 5.44 – 6.75), within further subgroup analysis; the median survival of Child-A patients was 7.4 months (95% CI: 5.08 – 9.78), while in Child-B was 3.6 months (95% CI: 2.73 – 4.43) with significant log rank value of (.002), and overall TTP was 4.9 months (95% CI: 2.55 – 7.24). CRP had a magnificent role in prediction of long term survival after treatment, CRP < 1.0 mg/l showed median survival of 15.2 months (95% CI: 8.1 – 22.3), and CRP > 1.0 mg/l had 4.9 months (95% CI: 3.8 – 5.9). Regarding the clinical AEs according to CTCv3, Grade 1/2 post-embolization fatigue syndrome and abdominal pain, mild nausea/vomiting and ascites were represented in majority of the patients after treatment. Biochemical AEs were more obvious among HCC patients with high CRP at baselines. **Conclusions:** It seems that C-reactive protein (CRP) has a magnificent role on prediction of survival outcome after radioembolization with Yttrium-90 microspheres. Also it could sort patient who are more susceptible for AEs after therapy. Further future evaluation versus molecular targeted therapy is warranted in protocols of clinical trials.

4.8 Predictors of Longer Survival after Radioembolization in Unilobar and Bilobar Advanced Hepatocellular Carcinoma with Yttrium-90

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Background: Hepatocellular carcinoma (HCC) is a common cancer that typically occurs in liver cirrhosis. Incidence is expected to increase dramatically in the next few decades. Less than 10% of newly diagnosed tumors outside of screening programs can be curatively resected. Sorafenib is the standard treatment for advanced stage HCC, however, there is to date no consensus on second line therapy and alternative treatment options. Radioembolization (RE) with Yttrium-90 microspheres is a novel transarterial treatment option for patients with advanced HCC. Careful patient selection is a key factor for success with RE. Hepatic functional reserve seems to be one of the most important variables to consider predictors of longer survival before treatment with radioembolization in advanced HCCs. **Patients and Methods:** During the time between November 2010 and March 2014, 202 consecutive patients with unresectable HCC were included either after TACE failure or with locally advanced HCC. Radioembolization with Yttrium-90 glass microspheres was performed in a lobar fashion through the right or left hepatic artery. In bilobar disease, right and left liver lobe were treated within 4 – 6 weeks intervals in between. The mean radiation dose was 127 (+/-29) Gy per treatment. Median survival time was considered as main endpoint with further subgroup clinical and biochemical analyses among unilobar and bilobar HCCs. **Results:** Among 202 patients with advanced HCC treated by 334 sessions of radioembolization using Yttrium-90 glass microspheres (mean: 1.5 sessions/patient). Unilobar advanced HCC was presented in 85 (42%) patients and bilobar advanced HCC in 117 (58%). In unilobar HCC, serum AST level and CRP displayed together important predictors for survival during baseline before treatment with radioembolization. In low AST & CRP 36/85 (42%) median survival was 20.1 months (95CI:14.5 – 25.6); in high AST & low CRP 3/85 (4%) was 15.3 months (95CI:10.5 – 19.8); in low AST and high CRP 38/85 (45%) was 9.0 months (95CI:5.2 – 12.7); and in high both AST & CRP 8/85 (9%) was 1.9 months (95CI:0.0 – 5.2) which predicts the worst survival possibility with P value < 0.001. On the other hand in bilobar HCC, multivariant analysis showed that BCLC stage and abdominal ascites had significant impact on long term survival. Thus, the median survival time in bilobar HCC in Child A patients in the absence of ascites, was 18.4 months (95CI:13.4 – 23.3) in BCLC-B with normal CRP versus 11.1 months (95CI:4.7 – 13.7) in high CRP patients; and was 8.5 months (95CI:5.4 – 11.7) in BCLC-C with normal CRP versus 5.8 months (95CI:3.8 – 7.7) in high CRP patients, with P value 0.01. CRP and AST were valuable predictors for better survival in unilobar HCC, while BCLC tumor stage and the presence of ascites in addition to CRP had shown significant influence on the overall survival in bilobar HCC. The main obvious adverse events were transient fatigue-syndrome without any clinical impact. Grade 3 – 4 biochemical toxicities occurred more obvious after the second treatment session in bilobar HCC. **Conclusions:** The number of HCC lesions, AST and CRP levels act as positive predictors for longer survival within unilobar HCC, while BCLC stage and ascites act as negative predictors in bilobar HCC.

4.9 Charakterisierung der Akut-Phase Reaktion in humanen Lebergewebekulturen mittels transkriptomweiter Expressionsanalysen

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Hintergrund: In Folge der metabolischen Überlastung der Leber und des Operationstraumas tritt nach ausgedehnter Leberteilresektion häufig ein postoperatives Leberversagen (POLF) auf. Dieses ist eine schwere Komplikation. Um den Beitrag genetischer Faktoren an dem interindividuell unterschiedlichen Entzündungsgeschehen, der mit dem POLF einhergeht, sollen eQTL- (Expression Quantitative Trait Locus) Analysen durchgeführt werden. Vorbereitend dazu wurde ein Gewebekulturmodell humaner Leberproben etabliert, das die durch nicht-sterile (z.B. LPS) oder durch sterile (z.B. pro-inflammatorische Cytokine) Stimuli hervorgerufene Entzündungsreaktion in der Leber in vitro imitiert. **Methoden:** Mit einem Tissue-Chopper wurden Gewebeschnitte (325 µm) aus humanen Leberteilresektaten angefertigt und mit einem Cocktail proinflammatorischer Zytokine (IL-6, IL1-β und TNFα) oder mit LPS stimuliert. Nach 8 h, 12 h und 24 h Inkubation wurde die Akut-Phase-Reaktion anhand des Anstiegs der Akut-Phase-Proteine CRP, SAA und Fibrinogen im Medienüberstand oder im Gewebe bestimmt. Zur Identifizierung differentiell exprimierter Gene wurden Array-basierte transkriptomweite Expressionsanalysen durchgeführt. **Ergebnisse:** Wahrscheinlich durch den operationsbedingten Gewebestress war die Akut-Phase-Reaktion in den Leberproben bereits ohne weitere Stimulation deutlich erhöht. Diese fiel im Verlauf der Gewebekultur ab. Durch die Stimulation mit Zytokinen bzw. LPS konnte die Sekretion der Akut-Phase-Proteine leicht gegenüber Ausgangswerten erhöht bzw. der Abfall im Kulturverlauf verlangsamt werden. Die Stimulation mit Cytokinen ergab im Vergleich mit LPS eine ca. 2,5-fach höhere Zahl an differentiell exprimierten Genen, von denen 20% durch beide Stimuli erhöht wurden. Durch Pathway-Analysen konnten die Gene u.a. inflammatorischen und immunmodulatorischen Signalwegen wie Cytokin- und Chemokin-Rezeptorinteraktionen sowie der hepatischen innaten Immunantwort, wie dem NOD- und Toll-like Rezeptor-Signaling, zugeordnet werden. **Schlussfolgerung:** In den Lebergewebekulturen konnte eine maximale Akut-Phase-Reaktion durch verschiedene inflammatorische Stimuli erzeugt werden. Transkriptomweite Expressionsanalysen lieferten signifikante Hinweise auf beteiligte Gene/Transkripte und Signalwege. Folglich eignet sich die Methode für zukünftige eQTL-Analysen zum Erfassen des genetischen Beitrags an der interindividuell unterschiedlichen Molekularpathogenese des POLF.

4.10 Combined Photodynamic Therapy with Systemic Chemotherapy Compared to Chemotherapy or Photodynamic Therapy Alone in Patients with Non-Resectable Extrahepatic Cholangiocarcinoma: a Retrospective Study

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Background: Photodynamic therapy (PDT) has been shown to be beneficial in patients with non-resectable extrahepatic cholangiocarcinoma. Systemic chemotherapy (SC) with gemcitabine and more recently with gemcitabine and cisplatin is the current first-line chemotherapy for advanced cholangiocarcinoma. This retrospective study analyzed for the first time a large cohort of patients with non-resectable extrahepatic cholangiocarcinoma treated either with combined PDT-SC, SC or PDT

alone. **Methods and patients:** 94 patients suffering from non-resectable extrahepatic cholangiocarcinoma were retrospectively analyzed. Patients were stratified according to treatment: systemic chemotherapy alone (SC, n = 29), PDT (n = 33) and a combination of both therapies (PDT-SC, n = 32). Chemotherapy regimens used were usually gemcitabine alone (1000 mg/m2 of body surface area) or in combination with cisplatin (25 mg/m2 of body surface area). **Results:** Median survival of patients treated with PDT-SC was significantly longer (19.5 months) followed by the PDT group (13 months) compared to the SC group (9 months), (p = 0.003 and p = 0.032 respectively) with a hazard ratio [HR] 0.488 (95% confidence interval [CI], 0.266 – 0.894). Grade 3 – 4 toxic adverse events were similar among the different groups. Tumor stage, presence of hepatopathy, history of a second malignacy and treatment method seemed to be prognostic factors with statistical significance in the univariate analysis. In the multivariate analysis, PDT-SC and PDT were significant independent predictors of longer survival. **Conclusion:** Combined PDT with a systemic chemotherapy was feasible, well-tolerated and resulted in significant longer overall survival than chemotherapy alone in patients with extrahepatic cholangiocarcinoma. Interestingly, also PDT alone improved survival compared to chemotherapy alone. Thus, prospective trials are warranted to further define the role of PDT in the standard palliative therapy of cholangiocarcinoma.

4.11 Current therapeutical options for hepatocellular carcinoma in a german liver center – initial results of a retrospective data analysis

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Background: Guidelines and recommendations as to how to treat HCC patients exist. Staging systems, i.e. the BCLC, have been created to support clinicians in finding the right individualized therapy. However, how does this translate into actual every day practice? Under which circumstances has resection been chosen as the first-line therapy? What are distinct characteristics and tumor features of resected patients as compared to those treated differently? To examine those questions it was essential to systemize and analyze available patient data. **Methods:** All patients discussed in the interdisciplinary liver board of the University Clinic Wuerzburg were screened. A total of 217 patients were included and analyzed. **Results:** 30% of patients underwent liver resection. Most of them in BCLC stage A (59%) but also in stage B and some even in stage C (almost 8%). They vary significantly from the non-resected patients as sorafenib treatment and best supportive care were reserved for later stages (BCLC C and D) (p = 0.03). However, distribution of the UICC and BCLC stages did not significantly differentiate between primary resection and primary TACE patients (over 90% of patients in both groups in BCLC A and B, p = 0.561). Nevertheless, a primary resection left significantly more patients free of any HCC recurrence than a primary TACE (48% and 15% respectively, p < 0.0001) but less than a primary transplantation (76%, p < 0,0001). No significant differences in the death rates were found between those three groups. **Conclusions:** No differences were detected in the number of deaths between resection and transplantation. Thus, resection can be seen as a legitimate alternative taking into account the scarce donor numbers. Both resection and interventional methods (TACE) are valid options in patients with early disease states. Still, resection appears to yield better long-term results as fewer patients suffered from relapsing HCC.

4.12 Cux1 confers resistance to apoptotic cell death in liver cancer cells

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Background and Aims: CUX1 (CUTL1) is a transcription factor able to promote the expression of several genes implicated in cellular proliferation, differentiation and demise. In normal adult cells, it preferentially favors the expression of proapoptotic genes. Its aberrant expression in tumor turns its role as foe. Here, we analyze CUX1 activity in TRAIL (Tumour necrosis factor related apoptosis inducing ligand) mediated cell death in liver cancer cells. **Methods:** CUX1 was knocked down in HepG2 and Hep3B cells. Cells were further treated for 48 hours with a strong ligand (superkiller) binding DR4 and DR5 (TRAIL death receptors). The cell death events were analyzed by FACS analysis. RT-qPCR was performed to detect the expression of apoptotic markers. Caspase activity was measured by luminescence. Apoptosis array was performed. **Results:** Treatment with superkiller TRAIL, at 50 and 100 ng/ml, caused cell death in HepG2 and Hep3B cells after 48 h proven by an accumulation of 40% of sub-G1 events. CUX1 knock down caused a sensitization of liver cancer cells to TRAIL effect by increasing, significantly, the percentage of sub-G1 events (60% with 100 ng/ml). CUX1 knock down did not change the expression of TP53, KRT18, CDKN1A and CDKN1B. Interestingly, silencing CUX1 increased the activity of caspase 3/7 after treatment with soluble TRAIL. The effect was neutralized by pan-caspase inhibitor zVAD. Apoptosis array evidenced an increased protein level of un-/cleaved caspase 3 after CUX1 knock down. **Conclusions:** CUX1 mediates the resistance of liver cancer cells to TRAIL signaling. Knock down of CUX1 restores the potential of TRAIL to trigger cell death.

4.13 CUX1 controls endoplasmic reticulum stress and autophagy related cell death

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Background and Aims: CUX1 (CUTL1) is a transcription factor able to promote the expression of several genes implicated in cellular proliferation, differentiation and demise. In normal adult cells, it preferentially favors the expression of proapoptotic genes. Its aberrant expression in tumor turns its role as foe. Here, we analyze the role exerted by CUX1 during deacetylase inhibitors mediated cell death in liver cancer cells. **Methods:** CUX1, endoplasmic reticulum (ER) stress and autophagy markers were analyzed by RT-qPCR in two liver cancer cell lines HepG2 and Hep3B. Protein level was measured by western blotting. Cells were transfected with siRNA for CUX1 and furthermore treated with deacetylase inhibitors panobinostat, SAHA and trichostatin A. Thapsigargin, an endoplasmic reticulum stress inducer, served as positive control. **Results:** CUX1 knock down caused a suppression of ER stress and autophagy markers BiP, CHOP, ATF4, ATF6, Beclin1, MAP1LC3B, UVRAG and TFEB at early time point (6 hours) in both cell lines. Prolonged transfection did not alter the expression of the above mentioned markers; BiP was the only one suppressed in HepG2 after 24 hours. Interestingly, the deacetylase inhibitors are able to promote CUX1 over-expression after 6 hours of treatment, whereas they show to lose this ability after 24 hours. CUX1 knock-down reduced not significantly its protein level after treatment with deacetylase inhibitors. CUX1 knock down counteracts the accumulation of BiP protein after 24 hours of treatment with deacetylase inhibitors. Thapsigargin induced BiP independently from CUX1. **Conclusions:** ER stress and autophagy markers are under the control of CUX1. The cell death induced by deacetylase inhibitors is strictly connected with CUX1 expression and activity. Further studies are needed to clarify the exact mechanism exerted by CUX1 in this scenario.

4.14 Cytokines and hepatocellular carcinoma: Potential progression markers and cell typespecific modulators of the mirnome (Interleukin-6 and JAK/STAT activating cytokines)

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HCC is the fifth most common and the third deadliest cancer worldwide. There is an urgent need to find sensitive markers for early diagnosis and to monitor postoperative recurrence in order to provide treatment for HCC. Cytokines play an important role in liver physiology, i.e., IL-6-type cytokines as mediators of the acute phase response. In addition, they

contribute to cancer development and progression. Thus, HCC-associated cytokines as well as miRNAs are candidates for diagnostic and prognostic biomarkers of HCC. **Methods:** So far, serum samples of 8 healthy controls, 23 nonalcoholic steatohepatitis (NASH) patients and 18 HCC patients have been analyzed regarding the levels of up to 48 cytokines with the Luminex immunoassay technology (Bio-Plex 2200, Bio-Rad). The effect of four cytokines: Hyper-IL-6 (H-IL-6), OSM, IFN-γ, and IL-27 on the miRNome and on the transcriptome of nine healthy and cancerous cell lines derived from human liver, colon and skin cells (PH5CH8, Hep3B, Huh-7; NCM460, HCT15, HCT116; NHEM, A375, MelJuso) has been analyzed by microarray (Affymetrix GeneChip miRNA Array 3.0, Human Transcriptome Array 2.0) and validated by qPCR. **Results:** In a first step, we analyzed and correlated the expression levels of inflammatory cytokines in HCC and NASH patients, being at high risk to develop HCC. Herein, serum levels of IL-6 and of HGF were higher in HCC patients than in healthy controls and in advanced NASH patients, as confirmed by Bio-Plex cytokine immunoassays. Next, we wanted to study a possible correlation between cytokine levels and the presence of the PNPLA3 p. 148 M risk variant in patients with NASH, thereby contributing to biomarker research in liver diseases. Next, we have studied the effect of IL-6 on the miRNome in hepatoma cell lines and primary hepatocytes. Surprisingly, IL-6 caused thousands of mRNAs to be differentially regulated in HepG2 and HuH-7 hepatoma cell lines, whereas levels of only few miRNAs were significantly changed (FDR < 0.05, logFC 0.5). In contrast to HCC cell lines, a stronger response of the miRNome following IL-6 stimulation was observed in primary hepatocytes (68 and 27 differentially expressed miRNAs in two samples, respectively). To further address this possible cell type and tissue specificity, we tested the effect of STAT-1 or STAT-3-activating cytokines (IL-27, IFN-γ, OSM and H-IL-6) on both the miRNome and the mRNA transcriptome of healthy and cancerous cell lines, derived from human liver, colon and skin cells. Moreover, the regulation and roles of selected IL-6/Jak/STAT3-related miRNAs with possible relevance for HCC are currently further analyzed. **Conclusions:** Our work may on one hand further help to correlate cytokine levels and the presence of a risk variant of pnpla3 in NASH patients, thereby contributing to biomarker research in liver diseases. On the other hand, we could participate to improve the knowledge on the question of the effect of cytokine stimulation on the miRNome and the transcriptome, in various cells and tissues cancerous or not.

4.15 Evaluation der pro-steatotischen Genvariante rs641738 im MBOAT7-Gen bei Patienten mit hepatozellulärem Karzinom (HCC)

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Einleitung: Der PNPLA3 (patatin-like phospholipase domain containing 3) Polymorphismus p.I148 M prädisponiert zur Fettleber und beeinflusst die Fibroseprogression bei Patienten mit chronischen Leberkrankheiten. Träger der Genvariante weisen zudem ein deutlich erhöhtes HCC-Risiko auf. Dies konnten wir kürzlich in einer Kohorte von 44 Patienten mit HCC und unterschiedlichen zugrunde liegenden Leberkrankheiten reproduzieren (Casper et al. Z. Gastroenterol 2016). Mit der MBOAT7-Genvariante rs641738 ist mittlerweile ein weiterer genetischer Risikofaktor für die Entstehung einer Steatose identifiziert worden (Mancina et al. Gastroenterology 2016). Ob Träger der Variante ebenfalls ein erhöhtes HCC-Risiko aufweisen, ist bislang noch nicht nachgewiesen worden. **Patienten und Methodik:** Untersucht wurden zwischen 01/2014 und 01/2016 47 prospektiv erfasste Patienten mit HCC (68 ± 10 Jahre; 74% Männer; 25 multifokale Tumoren, größter Herd 17 – 120 mm) und unterschiedlichen chronischen Leberkrankheiten (17 Alkohol; 10 Hepatitis C; 10 kryptogen, 5 Hepatitis B, 4 NAFLD, 1 AIH) sowie 245 Kontrollen ohne Lebererkrankung. Die MBOAT7-Variante wurde mittels Taqman-Assay genotypisiert. Die statistische Auswertung der Assoziation der Genvariante mit dem HCC erfolgte mithilfe des Armitage-Trend-Tests (Genotypen) und Vierfeldertafeln (Allele). **Ergebnisse:** Die MBOAT7-Variante konnte in allen Fällen ([CC] n = 12, [CT] n = 25, [TT] n = 10) und Kontrollen ([CC] n = 69, [CT] n = 117, [TT] n = 59) erfolgreich genotypisiert werden. Die Frequenz des Risikoallels betrug sowohl bei den Kontrollen als auch bei den Fällen 47%, sodass keine signifikante Assoziation zwischen der MBOAT7-Variante und dem HCC nachgewiesen werden konnte (common OR = 0,99, p = 0,98). Eine Abweichung vom Hardy-Weinberg-Äquilibrium konnte ausgeschlossen werden (p > 0,05 für Fälle und Kontrollen). **Schlussfolgerung:** In der saarländischen HCC-Kohorte konnte – im Gegensatz zu PNPLA3 – kein Effekt der MBOAT7-Variante rs641738 auf das HCC-Risiko nachgewiesen werden. Zum verlässlichen Ausschluss auch kleinerer Effekte der Variante auf das HCC-Risiko müssen im Verlauf weitere größere Kollektive untersucht werden.

4.16 Der SNP3099 im mTOR Gen ist prädiktiv für die Verträglichkeit und Wirkung von Everolimus nach Lebertransplantation

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Hintergrund: Die Verträglichkeit des mTor-Inhibitors Everolimus (EVR) als Immunsuppression (IS) nach Lebertransplantation (LT) ist individuell sehr unterschiedlich und möglicherweise auf Variationen im mTOR-Gen zurück zu führen. Um zu analysieren ob diese genetischen Veränderungen als Prädiktoren für das Auftreten von vermehrten Nebenwirkungen eingesetzt werden können, erfolgten SNP-Analysen an lebertransplantierten Patienten. **Material und Methoden:** Es wurden 127 lebertransplantierten Patienten, die auf eine EVR-basierte IS umgestellt worden waren, in diese Studie eingeschlossen, mit einem mittleren Alter zum Zeitpunkt der LT von 50 Jahren (2 – 67). Die Umstellung der Patienten auf EVR erfolgte durchschnittlich 1,5 Jahre (0,5 – 23) nach LT. Die SNPs 3099 und 8600 im mTOR-Gen wurden über Pyro-Sequenzierung untersucht und mit den klinischen Daten hinsichtlich des Auftretens von Nebenwirkungen, verschiedener Laborparameter sowie der Indikation zur Transplantation korreliert. Die statistische Auswertung erfolgte mittels Fischer-, χ2-Test oder ANOVA. **Ergebnisse:** Die SNP-Verteilung der Patienten stellte sich wie folgt dar: Im Falle des SNP3099 wiesen 45% der Patienten den CC, 45% den CG und 10% den GG-Genotyp auf. Beim SNP8600 wurden die Genotypen AA (93%) und AG (7%) nachgewiesen. Patienten mit einem C-Allel (CC/GC) in SNP3099 mussten die EVR-Therapie deutlich häufiger abbrechen (24%) als Patienten mit einem GG-Genotyp (kein Therapieabbruch). Patienten mit dem GG-Genotyp im SNP3099 zeigten zudem seltener einen Diabetes als Patienten mit einem A-Allel, entwickelten jedoch signifikant häufiger (p ≤ 0,024) einen de novo Diabetes (GG: 31%; CC: 9%; GC: 5%). Schlussfolgerungen: Der SNP3099 im mTor-Gen kann als Prädiktor für die Verträglichkeit der EVR-Therapie nach LT eingesetzt werden kann.

4.17 Die Inhibition von Polo-like Kinasen resultiert in Degradation von Bcl-2 und wirkt dadurch pro-apoptotisch in Cholangiocarzinom Zellen

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Das cholangiozelluläre Karzinom (CCC), eine nur limitiert behandelbare Tumorerkrankung der Gallenwege, ist in westlichen Ländern mit einer steigenden Inzidenz assoziiert. Die Tumorzellen weisen eine zunehmende Apoptose-Resistenz auf, die auf das Vorhandensein von effektiven Überlebenssignalen zurück zu führen ist und dadurch vermutlich die Therapierbarkeit des CCC erschwert. Interessanterweise sind CCC-Zellen gegenüber der Apoptose-Induktion durch den Todesliganden TRAIL (tumor necrosis factor-reated apoptosis-inducing ligand) unempfindlich, obwohl diese in-vivo sowohl TRAIL als auch dessen Todesrezeptor exprimieren. Die Heraufregulation anti-apoptotischer Proteine der Bcl-2 Familie könnte ein möglicher Mechanismus dafür sein. Zusätzlich potenziell wichtige Überlebenssignale, die allerdings bisher beim CCC noch unzureichend untersucht wurden, umfassen die Zellzyklus-regulierenden PLK (polo-like kinase) Signalkaskaden. Liegt bei Tumoren eine PLK-Überexpression vor, so ist dies mit einer schlechten Prognose assoziiert. Genauere mechanistische Erkenntnisse über anti-apoptotische Signalkaskaden können zur Entwicklung zielgerichteter Mechanismen-orientierter Therapien für diese verheerende Erkrankung beitragen. In dieser Studie untersuchen wir, welchen Einfluss die Inhibition von PLKs auf die Apop-

tose-Induktion sowie die Expression von Bcl-2 in CCC Zelllinien hat und welcher mögliche Therapie-unterstützende Effekt hiermit einhergeht. Dafür haben wir die CCC Zelllinien KMCH-1 und Mz-Ch-1 mit dem effektiven PLK-Inhibitor BI6727/Volasertib, dem Zytostatikum Cisplatin oder in Kombination beider Substanzen für 24 h inkubiert. Mithilfe eines MTT Assays wurde die Viabilität der Zellen bestimmt, die Anzahl apoptotischer Zellkerne wurde mithilfe floureszenzmikroskopischer Aufnahmen quantifiziert und die Aktivierung der Apoptose wurde durch einen Caspase-3/-7 Assay gemessen. Die Expression von Bcl-2 wurde durch Western Blot Analysen dargestellt. Die Behandlung mit dem PLK-Inhibitor BI6727 konnte in KMCH-1 und Mz-Ch-1 die zytotoxische Wirkung von Cisplatin verstärken. In einem weiteren Experiment konnte gezeigt werden, dass die Zugabe von BI6727 die Expression von Bcl-2 in Mz-Ch-1 Zellen verminderte. Schlussfolgernd verstärkte die Behandlung mit dem PLK-Inhibitor BI6727 die zytotoxische Wirkung von Cisplatin durch die Degradation des anti-apoptotischen Proteins Bcl-2 in CCC Zelllinien.

4.18 Distinct transcriptome and secretome patterns characterize capillarization in early and late phases of hepatocarcinogenesis

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Background: In chronic liver diseases and hepatocarcinogenesis capillary endothelial cells (CECs) gradually change the liver-specific microcirculation by replacing liver sinusoidal endothelial cells (LSECs). This process of capillarization impairs the hepatic function and is caused by micro-environmental changes such as hepatocyte secretome alterations. However, the impact of tumor-derived, secreted factors on capillarization as well as a comparative morphological, biochemical and molecular characterization of the involved endothelial cell types in different stages of hepatcarcinogenesis is missing. **Results:** The capillarization reaction was analyzed in transgenic mice with inducible and liver-specific expression of the constitutively active oncogene yes-associated protein (YAPS 127A; Tschaharganeh et al. 2013). Immunofluorescence of Lyve-1 and CD 146 revealed a progressive replacement of LSECs by CECs in livers, hyperplastic/premalignant lesions, and hepatocellular carcinomas (HCCs). FACS analysis of primary isolated endothelial cells confirmed the selective expansion of CECs with 4 ± 2% in normal liver, 37 ± 16% in hyperplastic livers, and 61 ± 30% in tumor-bearing livers. Expression profiling analysis of primary isolated LSECs and CECs from normal livers, premalignant lesions, and tumor-bearing livers identified stage- and cell type-specific molecular patterns including cytokine/chemokine signatures. To identify paracrine effector pathways regulating capillarization in tumorigenesis, proteome profiling of supernatants derived from primary hepatocyte- and YAPS 127A-induced tumor cells was performed. Several proangiogenic factors were induced in tumor cells including the placental growth factor (PGF) and tissue inhibitor of metalloproteinase 1 (TIMP1), illustrating an oncogene-dependent paracrine effect on endothelial cells. Analysis of human HCC gene expression data from 242 HCC patients confirmed the prognostic relevance of the identified proangiogenic factors (Roessler et al. 2010). **Conclusion:** Capillarization is a specific event in hepatocarcinogenesis characterized by distinct molecular traits. Dynamic changes of the hepatocellular secretome occur early in liver tumor development and its perturbation might represent a possible approach for an early inhibition of tumor-supporting capillarization.

4.19 Einfluss des Sozialstatus auf Diagnosestellung und Therapieverlauf bei HCC am Universitätsklinikum Hamburg-Eppendorf 2008 bis 2012

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Einleitung: Der Einfluss des Sozialstatus auf Diagnosestellung und Therapieverlauf ist bei Patienten mit hepatozellulärem Karzinom (HCC) bisher unzureichend untersucht. **Ziele:** Das Ziel dieser Arbeit ist, den Zusammenhang zwischen Sozialstatus und Ätiologie, Diagnosestellung und Therapieverlauf bei HCC in unserem Patientenkollektiv darzustellen. **Methoden:** Retrospektiv wurden Daten von 607 Patienten, die zwischen 2008 und 2012 am Universitätsklinikum Hamburg-Eppendorf aufgrund eines HCC eine Therapie erhielten, ausgewertet. Die Ermittlung des Statusindex (SI) der in Hamburg gemeldeten Patienten (n = 301) erfolgte auf der Basis von Angaben der Behörde für Stadtentwicklung und Umwelt der Freien und Hansestadt Hamburg. **Ergebnis:** Die Verteilung der SI der in Hamburg wohnhaften Patienten mit HCC entsprach der Verteilung der SI innerhalb der Hamburger Gesamtbevölkerung (n = 1732144), d. h. es gab keine Häufung des HCC in bestimmten SI-Gruppen in unserem Patientenkollektiv (p = 0,700; Abbildung 1). Wie erwartet hatten Patienten mit niedrigem SI im Vergleich zu Patienten mit höherem SI häufiger eine Hepatitis B als Risikofaktor (OR: 4,5; CI: 1,21 – 16,69). Bei den weiteren Risikofaktoren (Alkohol, Hepatitis C) ergab sich zwischen den einzelnen SI-Gruppen kein signifikanter Unterschied. Patienten mit hohem SI waren zum Zeitpunkt der Diagnosestellung älter (p = 0,017) und zeigten im Vergleich zu Patienten mit niedrigem SI keinen signifikanten Unterschied in der mittlere Überlebenszeit (36,3 vs. 23,1 Monate; p = 0,283). **Schlussfolgerung:** In unserem Patientenkollektiv zeigte sich kein Unterschied in der Verteilung des Sozialstatus zu der Hamburger Gesamtbevölkerung. Die mittlere Überlebenszeit ist zwischen Patienten mit hohem und niedrigem SI nicht signifikant abweichend.

4.20 Evaluation der Serumkonzentration von Osteopontin als diagnostischer und prognostischer Biomarker in Patienten mit cholangiozellulärem Karzinom

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Einleitung: Die Überexpression von Osteopontin, einem sezernierten extrazellulären Glykoprotein, wurde in verschiedenen Tumorentitäten wie dem hepatozellulärem Karzinom, dem Pankreaskarzinom sowie dem Magenkarzinom nachgewiesen. Beim cholangiozelluären Karzinom (CCC) wurden widersprüchliche Ergebnisse hinsichtlich der Expression von Osteopontin und dessen klinischer Relevanz beschrieben. Für verschiedene gastroenterologische Tumore, jedoch nicht für das CCC, wurde die Serumkonzentration von Osteopontin als möglicher diagnostischer und prognostischer Biomarker diskutiert. In dieser Studie evaluieren wir eine potentielle Funktion von Osteopontin als neuartiger Serumbiomarker. **Methoden:** Die mRNA-Expression von Osteopontin (SPP1) wurde in humanen und murinen CCC-Gewebeproben mittels qPCR analysiert. Serumkonzentrationen von Osteopontin wurden mittels ELISA in 79 Patienten mit histologisch nachgewiesenem CCC, die sich einer chirurgischen Resektion unterzogen, bestimmt und mit 42 gesunden Kontrollen verglichen. Die Ergebnisse wurden mit klinischen Daten korreliert. **Ergebnisse:** Die Gewebsexpression von Osteopontin war in murinen und humanen CCC Proben deutlich gesteigert. Weiterhin zeigten CCC-Patienten im Vergleich zur Kontrollgruppe signifikant erhöhte Serumkonzentrationen von Osteopontin. Patienten mit metastasiertem CCC wiesen im Vergleich zu Patienten ohne Fernmetastasen höhere Osteopontinserumkonzentrationen auf. Die Cox-Regression und Kaplan-Mey-

er-Kurvenanalyse zeigten, dass präoperative und insbesondere postoperative Serumkonzentrationen von Osteopontin mit dem Gesamtüberleben der Patienten korrelieren. **Schlussfolgerung:** Die Expression von Osteopontin ist in murinen und humanen CCC-Gewebeproben gesteigert. Zudem stellt die Analyse der Serumkonzentration von Osteopontin in CCC-Patienten einen vielversprechenden diagnostischen und prognostischen Biomarker dar.

4.21 Evaluation of a system for normothermic and subnormothermic ex vivo machine perfusion of isolated rat livers

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Background: Poor patient outcome of extended criteria donor (ECD) liver grafts after static cold storage calls for new methods of ex vivo organ preservation in light of the exhausted donor organ pool. In recent years, ex vivo machine perfusion at different temperatures, ranging from hypothermic (4 °C), subnormothermic (21 °C) to normothermic (37 °C), reemerged as possible alternative to static cold storage. Our aim was to establish and evaluate an ex vivo perfusion system for rat livers that mimics the clinical conditions of machine-perfusion. **Methods:** We investigated perfusion conditions at both subnormothermic and normothermic temperatures with varying setups. The perfusion system consists of a pressure-controlled roller pump, an oxygenator, and a custommade perfusion chamber. Male Wistar rat livers were perfused via the portal vein for 12 hours using solely oxygenated culture medium or oxygenated culture medium (DMEM) supplemented with rat erythrocytes. A final perfusion setup using erythrocyte supplemented culture medium and an added circuit connected via a dialysis membrane for plasma expansion was established. **Results:** Potassium, Glucose and Urea concentractions soon reached high levels with solely oxygenated and erythrocyte-supplemented medium. The addition of the dialysis circuit led to almost physiological electrolyte concentrations in the perfusate with lower transaminase secretion during perfusion. Normothermic perfusion showed a greater Transaminase release compared to subnormothermic perfusion but also increased bile and Urea production, indicating a more active metabolism. Histological analysis, using HE and TUNEL-stains, of perfused rat livers showed no differences between both temperatures and showed an intact vascular system and lobular structure. **Conclusion:** As observed in normothermic human liver machine perfusion, plasma expansion was key for maintaining physiological perfusion conditions during perfusion. Our system might be suitable for small animal studies of machine perfusion of the liver at both temperatures.

4.22 Ginkgo biloba induces different gene expression signatures of oncogenic pathways in malignant and non-malignant cells in the liver

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Background: The Ginkgo biloba extract, EGb761, is a herbal supplement obtained from the leaves of the ginkgo tree with diverse biological properties. Recent studies indicate that EGb761 confers both preventive effects as well as anti-tumor effects in a variety of tumors, including hepatocellular carcinoma (HCC). We here evaluate the functional and mechanistic effects of EGb761 on human hepatocellular carcinoma cells as well as untransformed hepatocytes. **Methods:** Human hepatoma cell lines, primary human HCC cells and immortalized human hepatocytes were exposed to various concentrations (0 – 1000 ug/ml) of EGb761. Effects on proliferation and apoptosis were evaluated after 72 h of EGb761 exposure. Molecular changes were assessed by gene expression microarrays and western blotting. **Results:** EGb761 administration significantly impaired proliferation and induced apoptosis in hepatoma cells as well as hepatocytes. Median IC 50 for the hepatoma cells was dramatically lower than in hepatocytes suggesting a different response of EGb761 on normal and malignant cells. Consistently, while EGb761 induced a significant reduction in both colony and sphere forming ability in hepatoma cells, the treatment caused no mentionable changes in untransformed cells. Global gene expression analyses identified different gene expression signatures by EGb761 treatment in each cell line. Transcriptomic changes predominantly affected key oncogenic properties resembling in cell growth and proliferation as well as NRF2-mediated oxidative stress response in hepatoma cells. Furthermore, comparative analyses of the affected molecular pathways in hepatoma cells and hepatocytes identified a differential regulation of MAPK/ERK and PI3K/AKT/mTOR signaling. In consequence, regulation of eIF4 and p70SK6 was affected in hepatoma cells possibly leading to a disruption of cell growth by impaired protein biosynthesis. **Conclusion:** EGb761 differently affects hepatocytes and human hepatoma cells by modulating oncogenic pathways. While anti-tumorigenic and pro-apoptotic changes were induced in hepatoma cells, untransformed cells remained unaffected suggesting that EGb761 could be safely used for both preventive as well as therapeutic strategies.

4.23 Guanine monophosphate synthetase (GMPS) is an important target of p53-mediated repression in liver cancer

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Upon different types of stress the tumor suppressor protein p53 mediates a variety of cellular outcomes such as cell cycle arrest, apoptosis, and senescence by activation or repression of its target genes. How metabolic alterations either initiate or be part of the p53 response has been actively studied. However, de novo purine biosynthesis remains one of the few essential metabolic pathways for which a regulation by p53 is still poorly understood. Here, by using a large scale proteomics approach (LC/MS-MS) we identified guanine monophosphate synthetase (GMPS), a key enzyme of the purine biosynthesis pathway, as a previously unrecognized p53 repression target. Repression of GMPS by p53 could be validated by immunoblotting in Sk-Hep1, HepG2, and HuH6 cells. Furthermore, we found GMPS to be transcriptionally repressed in a p21-dependent fashion and its repression to be maintained in the context of p53-mediated cellular senescence. Notably, direct depletion of GMPS by RNAi was sufficient to trigger cellular senescence. Consistent with these findings we observed significant overexpression GMPS in murine and human HCCs compared to non-tumorous liver tissues. Importantly, particularly high GMPS expression was found in murine HCCs which developed in a p53 -/- background (compared to those with a p53 +/+ background). These data suggest that repression of GMPS is a relevant part of the p53-mediated senescence program representing a novel link between de novo purine biosynthesis and p53-related tumor suppression in liver cancer.

4.24 Hypoxia causes down-regulation of Dicer in hepatocellular carcinoma, which is required for up-regulation of hypoxia inducible factor 1α and epithelial-mesenchymal transition

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Purpose: A role of Dicer, which converts precursor miRNAs to mature miRNAs, in the tumor-promoting effect of hypoxia is currently emerging in some tumor entities. Its role in hepatocellular carcinoma (HCC) is unknown. **Experimental design:** HepG2 and Huh-7 cells were stably transfected with an inducible Dicer expression vector and were exposed to hypoxia/normoxia. HepG2-Dicer xenografts were established in nude mice; hypoxic areas and Dicer were detected in HCC xenografts and HCCs from mice with endogenous hepatocarcinogenesis; epithelial mesenchymal transition (EMT) markers were analyzed by immunohistochemistry or by immunoblotting. The correlation between Dicer and carbonic anhydrase 9 (CA9), a marker of hypoxia, was investigated in resected human HCCs. **Results:** Hypoxia increased EMT markers in vitro and in vivo and led to a downregulation of Dicer in HCC cells. The levels of Dicer were down-regulated in hypoxic tumor regions in mice with endogenous hepatocarcinogenesis and in HepG2 xenografts. In human HCCs the levels of Dicer correlated inversely with those of CA9, indicating that the negative regulation of Dicer by hypoxia also applies to HCC patients. Forced expression of Dicer prevented the hypoxia-induced increase in hypoxia inducible factor 1α (HIF-1α), hypoxia-inducible genes (CA9, glucose transporter 1), EMT markers and cell migration. **Conclusions:** We here identify down-modulation of Dicer as novel essential process in hypoxia-induced EMT in HCC and demonstrate that induced expression of Dicer counteracted hypoxia-induced EMT. Thus, targeting hypoxia-induced down-modulation of Dicer is a promising novel strategy to reduce HCC progression.

4.25 IGFBP2 – a novel p53-family target gene in hepatocellular carcinoma

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Background: In a variety of tumors, among these hepatocellular carcinoma (HCC), p53 family members exert cancerogenic or tumorsuppressive effects. p53 transcription factors (p53, p63, p73) react to cellular stress by transcriptionally regulating a specific set of genes. Target genes can be activated or inhibited depending on the particular p53 splice variants – with transactivation domain (TA) or dominant negative (DN) – and the characteristics of the specific binding site. We previously identified IGFBP2 (Insulin-Like Growth Factor Binding Protein 2) as a putative p53-protein target gene with prognostic relevance in HCC. The aim of this study was to elucidate how IGFBP2 is regulated by p53 family members. **Methods:** Hep3B cells were transfected with rAd-p53 and -TAp73. Transcriptional regulation of IGFBP2 was determined by qPCR. Western Blot and ELISA were used to investigate intra- and extracellular protein levels of IGFBP2. Transfac database analysis was performed to identify potential p53-family binding sites in the IGFBP2 locus. These sequences were then cloned, mutated and evaluated by luciferase reporter assays to confirm p53 binding. **Results:** TAp73-transfection induced a more than 25-fold increased IGFBP2 expression. A remarkable increase in intra- and extracellular IGFBP2 protein levels after TAp73-transfection was measurable as in controls no IGFBP2 was detectable. Transfection with TAp53 resulted in an up to 7-fold increased IGFBP2 expression. Two putative p53 and p73 binding sites (BS) are located within the promoter region. Another five putative BS for p73 and one for p53 were identified within intron 1 of the IGFBP2 locus. In TAp73-transfected cells intron1-dependent luciferase activity was increased by up to 150-fold, in TAp53 transfected cells by up to 20-fold. The identified p53 BS in intron 1 was confirmed, since after mutation and deletion of the latter luciferase activity was reduced by up to 90% in TAp53 and TAp73 transfected cells. Deletion of one of the putative p73 BS resulted in a reduction of luciferase activity by 85% after TAp73 transfection. **Conclusion:** These results clearly identify IGFBP2 as a novel target gene for TAp73 and TAp53 in HCC. By demonstrating for the first time the interaction between TAp73 and IGFBP2 signaling we succeeded in finding an important, formerly unknown, link between p53-family network and the IGF axis. p53-family members exert tumor-inhibiting effects whereas the IGF axis is of crucial importance for cell proliferation. It is suggestive that the balance of these two signaling pathways influences tumor characteristics as well as treatment response. Therefore, apart from enhancing our understanding of cancerogenic processes in HCC our results indicate that fine-tuning of these pathways might offer new therapeutic options in HCC, an entity with so far limited therapeutic measures.

4.26 Improved perioperative management using LiMAx test in liver surgery – preliminary results from the randomized Fast-track LiveR trial

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Background: The LiMAx test can validly determine liver capacity and has been successfully integrated in clinical management in liver surgery. However, no prospective randomized trials have been available to judge its actual clinical impact. **Methods:** A randomized controlled trial (RCT) was conducted during January 2013 to September 2015 in six recruiting clinics. Patients prior open liver resection of at least one segment were included. Patients were randomly assigned to LiMAx group (pre-, and postoperative LiMAx test) or control group (standard-of-care). Stable patients with sufficient residual liver function (LiMAx > 150 µg/kg/h) were directly transferred to general ward after surgery. **Results:** A total of 148 patients were randomized. Patients in LiMAx group were more frequently directly transferred to general ward after surgery (62.1% vs. 1.7%; $p < 0.0001$), the risk of severe postoperative complications was lower (grade IIIa; 14% vs. 28%; $p < 0.02$) and the length of post-operative stay was shorter (10 vs. 13 days; $p = 0.01$). No patient in LiMAx group was admitted to intensive care after primary transfer to general ward. **Conclusion:** The LiMAx test distinctively improves perioperative management in liver surgery. The valid identification of low risk patients skipping intensive care enables enhanced recovery.

4.27 Kaltlagerung und Kryokonservierung primärer humaner Hepatozyten in modifizierter TiProtec®-Lösung

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Primäre humane Hepatozyten werden in der pharmakologischen und toxikologischen Forschung sowie für die Zelltransplantation benötigt. Um die Verfügbarkeit der Zellen sicherzustellen, ist es notwendig, geeignete Methoden der mittel- und langfristigen Lagerung zu entwickeln. Allerdings sind die Zellen sehr empfindlich gegenüber Kälte- und Kryokonservierungsschädigung. Basierend auf der von uns entwickelten Ge-

webelagerungslösung TiProtec wurden verschiedene modifizierte Lösungen für die Kaltlagerung und Kryokonservierung humaner Hepatozyten getestet. Humane Hepatozyten in Suspension (10^6 Zellen/ml) wurden in serumfreier, modifizierter TiProtec oder Zellkulturmedium (Williams' E mit 10% Serum und weiteren Zusätzen) bei 4 °C kalt gelagert bzw. kryokonserviert. Für die Kryokonservierung wurde den Lösungen 10% DMSO zugesetzt und die Zellen mit -1 °C/min in einem Controlled Rate Freezer eingefroren. Nach Kaltlagerung bzw. schnellem Auftauen wurden die Zellsuspensionen ohne weitere Aufreinigung auf kollagenbeschichteten Zellkulturplatten ausgesät. Anheftungsrate und metabolische Aktivität wurden bestimmt und auf Kontrollkulturen der jeweiligen Isolierung bezogen. Nach Kaltlagerung in Zellkulturmedium sank die Anheftungsrate nach 24 h auf 51 ± 15%, nach 48 h auf 22 ± 12%. Durch Kaltlagerung in modifizierter TiProtec (chloridarm, erhöhte Eisenchelatorkonzentration) konnte die Anheftung nach 24 h Lagerung signifikant auf 92 ± 15% verbessert werden. Auch nach 48 h lag die Anheftung noch bei 63 ± 9% der frischen Kontrollzellen. Nach Kryokonservierung konnten Anheftungsrate (25 ± 29% vs. 14 ± 11%) und metabolische Aktivität (41 ± 35% vs. 23 ± 11%) durch die Verwendung einer chloridarmen TiProtec-Variante im Vergleich zu Zellkulturmedium ebenfalls deutlich verbessert werden. Zusammenfassend stellen chloridarme TiProtec-Varianten eine gute serumfreie Alternative für die Kaltlagerung und Kryokonservierung humaner Hepatozyten dar.

4.28 Kryokonservierung von Rattenhepatozyten mit einer neuen Einfrier- und Rekultivierungslösung

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Hepatozyten sind relativ empfindlich gegenüber Kryokonservierungsschädigung, insbesondere im 3D-Modell adhärenter Kulturen. Die Zellqualität nach Kryokonservierung wird, wie wir kürzlich zeigen konnten, neben physikalischen Parametern und dem Kryoprotektivum, auch von der Zusammensetzung der Einfrierlösung beeinflusst. Daher haben wir die Einfrierlösung für Hepatozyten optimiert und eine Rekultivierungslösung entwickelt. Adhärente primäre Rattenhepatozyten oder Hepatozytensuspensionen wurden in Zellkulturmedium oder Varianten einer neuen serumfreien Einfrierlösung, jeweils mit 10% DMSO, eingefroren. Der Anteil vitaler adhärenter Zellen wurde direkt nach dem Auftauen und nach Rekultur, die metabolische Aktivität (Resazurinreduktion) nach Rekultur bestimmt. Nach Kryokonservierung adhärenter Rattenhepatozyten in Zellkulturmedium verblieben 45 ± 9%, nach anschließender Rekultur (4 h) in Zellkulturmedium 8 ± 3% vitale Zellen im Vergleich zu nicht eingefrorenen Kontrollkulturen. Die metabolische Aktivität betrug 15 ± 8%. Das Einfrieren in der optimierten Lösung mit makromolekularem Zusatz verbesserte die Vitalität direkt nach dem Auftauen erheblich (> 95% vitale Zellen), allerdings kam es immer noch zu massivem Zelltod in der Rekultur. Durch Einsatz der neuen Einfrierlösung und einer angepassten Rekultivierungslösung konnte der Anteil lebender Zellen nach 4 h Rekultur auf 55 ± 2% und die metabolische Aktivität auf 74 ± 10% signifikant verbessert werden. Nach Einfrieren in Suspension konnte der Anteil vitaler, angehefteter Zellen nach Aussäen und 24 h Kultur von 15 ± 8% (Zellkulturmedium) auf 56 ± 17% (neue Lösung) erhöht werden. Der protektive Effekt in Zellsuspensionen blieb auch bei Verwendung von 5 und 10% Glycerin statt 10% DMSO erhalten. Zusammenfassend kann die Vitalität und die metabolische Aktivität von Rattenhepatozyten durch Einsatz optimierter Einfrier- und Rekultivierungslösung signifikant verbessert werden.

4.29 Loss of APC/CDH1 confers sensitivity to synthetic endoplasmatic reticulum stress through the dysregulation of GADD34

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Introduction: Endoplasmatic reticulum (ER) stress is a cellular response mechanism which allows a cell to react to protein overload, hypoxia, and nutrient deprivation. Cancer cells are especially prone to these types of challenges as they frequently encounter cellular stress due to unfavourable environmental conditions or as a result of antitumoral treatments. GADD34 is a protein which is highly expressed in cells undergoing ER stress, and its involvement in the restoration of homeostasis or the induction of apoptosis under unresolvable stress puts it into the focus of this project. We aim to explore whether synthetic lethal ER stress may be exploited in liver cancer therapy in the future, especially for patients which suffer from tumor entitites with intrinsically disturbed ER stress responses. **Methods and Results:** Using two hybrid screening in yeast, we identified GADD34 as a substrate of the Anaphase Promoting Complex/Cyclosome-CDH1 complex (APC/CDH1). We found that the APC/CDH1 mediated ubiquitylation of GADD34 favors survival in cells undergoing proteotoxic stress. Conversely, the loss of CDH1 sensitized stressed cells to GADD34 induced cell death. Importantly, we showed that cancer cells with reduced intrinsic activity of APC/CDH1 as caused by the loss of the tumor suppressor PTEN were hypersensitive to ER stress. **Summary:** Together our results reveal a new mechanism of GADD34 turnover and provide evidence for a synthetic lethal combination between the reduction in APC/CDH1 activity and sensitivity to ER stress.

4.30 Major Histocompatibility Complex (MHC) Class I-associated phosphopeptides as potential targets for immunotherapy in hepatocellular carcinoma (HCC)

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Despite the high incidence of HCC, being the fifth most common cancer worldwide, current therapeutic options remain limited for advanced HCC. There is, however, first evidence of clinical improvement after checkpoint blockade and it has also been shown that CD8+ T-cell responses targeting tumor antigens beneficially impact the survival of HCC patients. Immunotherapy therefore seems to be a promising approach for HCC treatment. The identification of tumor-specific antigens provides the basis for the development of an efficient targeted immunotherapy. Of note, until today only few tumor-specific antigens for HCC were identified. Promising proteins that harbor tumor antigens are so called phosphoproteins. This is because phosphoproteins are abundantly integrated in most signaling pathways and dysregulation of signaling pathways including aberrant and increased phosphorylation of proteins represents one hallmark of cancer. These emerging phosphoproteins can be degraded and the derived peptide fragments are presented via MHC-class I molecules on the cell surface of altered cells leading to T-cell recognition. We therefore claim that MHC-class I-associated phosphopeptides are attractive new tumor antigens for CD8+ T-cell-based immunotherapy of HCC. In order to identify tumor-associated phosphopeptides, to understand their significance for tumor immunity and to evaluate whether they could serve as potential tumor-specific antigens in HCC, HCC and adjacent liver tissues were lysed and MHC-class I peptide complexes were affinity-isolated. Subsequently, phosphopeptides were enriched and sequenced, using mass spectrometry. Interestingly, most of the identified phosphopeptides were displayed by Human Leukocyte Antigen (HLA)-B7 or HLA-B27 molecules and nearly 100 HLA-B7-associated phosphopeptides were identified. HLA-class I-presented phosphopeptides were found predominantly on cirrhotic liver tissue and HCC when compared to healthy tissue. Notably, many of the underlying proteins play an important role in tumor progression or survival, making them especially interesting for immunotherapeutic strategies. Based on their potential involvement in tumor progression, 21 phosphopeptides were further selected for immunological testing. Peripheral blood lymphocytes from healthy individuals, patients with chronic liver diseases or HCC patients were isolated and stimulated with the phosphopeptides for 7 days followed by intracellular cytokine staining. CD8+ T cell responses against this novel class of tumor antigens were comparable in quantity and quality to those seen against viral epitopes. Phosphopeptide-specific CD8+ T-cell responses were found in patients with chronic liver disease and liver cirrhosis. Our results therefore suggest that MHC-class I-presented phosphopeptides may be the target of cancer immune surveillance in liver disease and therefore represent an attractive target for future cancer immunotherapies of HCC.

4.31 Mesenchymale Stammzellen vermindern die durch Diethylnitrosamin induzierte Entstehung prä-neoplastischer Foci in der Mausleber

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Hintergrund: Nach wie vor stehen mesenchymale Stammzellen (MSC) im Verdacht, die Tumorgenese zu stimulieren oder selbst Tumore auszubilden. Da MSC als vielversprechende Option zur Therapie bei akuten und chronischen Lebererkrankungen gelten, sollte untersucht werden, ob die durch Diethylnitrosamin (DEN) induzierte Entstehung von Lebertumoren in der Maus durch MSC gefördert wird. **Methoden:** Pfp/Rag2-/-Mäusen wurde am 12. Lebenstag einmalig 20 µg/kg Körpergewicht DEN i. p. injiziert. Humane GFP-markierte MSC des subkutanen Fettgewebes (0,75 × 106 Zellen) wurden 7, 14 und 20 Wochen nach DEN-Gabe über die Schwanzvene systemisch appliziert. 24 Wochen nach DEN-Gabe wurden FAH (foci of altered hepatocytes), die als präneoplastische Läsionen gelten, in HE-Schnitten ausgezählt. **Ergebnisse:** Nach 24 Wochen induzierte DEN signifikant die Ausbildung von FAH. Die Applikation von MSC 14 oder 20 Wochen nach DEN-Gabe hatte darauf keinen signifikanten Einfluss. Wurden die MSC jedoch einmalig 7 Wochen, 2-malig nach 7 und 14 Wochen oder 3-malig nach 7, 14 und 20 Wochen verabreicht, waren keine FAH mehr nachweisbar. Diese Wirkung erfolgte parakrin durch bisher nicht näher identifizierte Faktoren, da unter keinen Umständen MSC oder von MSC abgeleitete Tumore in der Leber nachweisbar waren. **Schlussfolgerung:** Über parakrine Mechanismen hemmten MSC die Tumorgenese zu einem frühen Zeitpunkt. MSC selbst schienen keine Tumore zu bilden. Man kann daher annehmen, dass MSC zur Therapie während der Prä-Kanzerogenese, nicht jedoch zur Therapie bei Lebertumoren geeignet sind.

4.32 Verbesserung der Leberfunktion durch mesenchymale Stammzellen im Mausmodell des Alpha-1-Antitrypsinmangels

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Hintergrund: Die Therapie monogenetischer Erkrankungen der Leber durch die Transplantation von aus Stammzellen abgeleiteten hepatozytären Zellen gilt als vielversprechend. Der Alpha-1-Antitrypsin (AAT)-Mangel ist für die Leber eine ernsthafte Komplikation, da Alpha-1-Antitrypsin-Ablagerungen chronisch zur Leberzellschädigung und Leberzirrhose führen können. In der vorliegenden Arbeit wurde das therapeutische Potential von mesenchymalen Stammzellen (MSC) aus dem Fettgewebe der Maus hinsichtlich der Verbesserung der Leberfunktion im Mausmodell des AAT-Mangels (PiZ) untersucht. **Methoden:** MSC wurden aus dem Fettgewebe von männlichen Pfp/Rag2-/-Mäusen isoliert und entweder undifferenziert belassen oder für 14 Tage in hepatozytäre Zellen differenziert. Nach 1/3-Hepatektomie wurden 0,8 × 106 Zellen intrasplenisch in weibliche oder männliche PiZ-Mäuse injiziert. PBS diente der Kontrollinjektion, gänzlich unbehandelte Tiere als Referenz. Nach 1, 3 und 7 Wochen wurden Serum- und Leberproben entnommen und analysiert. **Ergebnisse:** Die MSC hatten keinen nennenswerten signifikanten Einfluss auf den Allgemeinzustand der Tiere oder die Leberregeneration wie die Bestimmung des Körpergewichts und des Leber-Körpergewichtverhältnisses ergaben. Die Bestimmung des Anteils transplantierter Zellen an der Gesamtlebermasse durch XY-Screening in weiblichen Tieren ergab, dass sich der Anteil der transplantierten Zellen im Zeitraum von 1 – 3 Wochen annähernd verdoppelte (von 1 auf ca. 2 – 3 %), danach aber wieder abnahm. Die Messung der Transaminasen im Serum ergab keine auf die MSC-Behandlung zurückzuführende Verbesserung, wohl aber wurden die Albuminspiegel in männlichen Tieren durch die Transplantation von MSC signifikant erhöht. Histologisch waren keine Unterschiede feststellbar. **Schlussfolgerung:** MSC verbesserten die Leberfunktion im Modell des AAT-Mangels. Dieser Effekt scheint transient, da keine signifikanten histologischen Verbesserungen festzustellen waren. Weiterhin scheint es geschlechtsspezifische Wirkungsunterschiede zu geben.

4.33 Non-invasive HCC diagnosis in daily practice

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Studies on the performance of non-invasive hepatocellular carcinoma (HCC) diagnosis are mainly performed under controlled conditions, which might not be met in clinical practice. We retrospectively analyzed all patients, in which a CT-guided liver biopsy was performed in a tertiary referral center within a 9 year period due to suspected HCC. The performance of non-invasive HCC diagnosis (incl. the Liver Imaging Reporting and Data System (LI-RADS)) was assessed. There was no significant difference in diagnostic performance between CT, conventional MRI, and Gd-EOB-DTPA-MRI. Late arterial phase images, which had a significant higher sensitivity for HCC detection compared to early arterial phase images, were obtained in less than 50 % of patients. The number of false positive non-invasive HCC diagnosis was higher in non-cirrhotic compared to cirrhotic patients (n = 13/19), while 19 out of 22 false-negative HCC diagnosis occurred in cirrhotic patients. Overall, the performance of LI-RADS to detect HCC was significantly better compared to AASLD and EASL-EORTC guidelines. Bleeding complications were observed in 3 % of patients, all could be managed conservatively and biopsy-related mortality did not occur. Needle tract seeding was not reported in the files. HCC biopsy is a reliable and safe procedure. Histological validation of non-invasive HCC diagnosis seems always warranted, when the radiological hallmarks are not fully met or the observation was made in a patient being low-risk for HCC development. Adjustment of examination protocols (e. g. bolus tracking for the timing of the arterial phase) may help to evaluate focal liver lesions in a standardized fashion. The use of LI-RADS is encouraged in clinical practice, as the differential diagnosis of HCC, ICC, and cHCC-CC has profound clinical implications and LI-RADS seems to have the power to separate these entities better than classical algorithms for non-invasive HCC diagnosis.

4.34 Oncogenic transcriptional co-activators YAP and TAZ regulate the expression of MCM helicase constituents in hepatocellular carcinoma

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Overexpression of the Hippo pathway-regulated transcriptional co-activator Yes-Associated Protein (YAP) is frequently detected in human hepatocellular carcinoma (HCC) and correlates with poor clinical outcome of cancer patients [1]. In mice, expression of the constitutively active isoform YAP S127A induces hepatomegaly due to the induction of hepatocellular proliferation followed by tumor formation within 12 weeks. However, how oncogene YAP induces uncontrolled proliferation in liver cells is not known, yet. In addition, the potential synergistic function of TAZ (syn. WWTR1, WW Domain Containing Transcription Regulator 1), another Hippo pathway downstream effector, has not been defined so far. Analyses of expression profiling data, derived from human liver cancer cells after siRNA-mediated inhibition of endogenous YAP, revealed that the Minichromosome Maintenance Complex Components (MCMs) family members MCM2 – 7 were positively regulated by YAP. MCMs are part of the pre-replication complex and are involved in the formation of the replication fork, which is essential for efficient DNA duplication followed by mitosis [2]. We confirmed that silencing of YAP by independent siRNAs reduced the expression of MCM2 – 7 at the transcript and MCM2 and MCM7 at the protein levels in different liver cancer cell lines. Interestingly, first in vitro data demonstrated that both, YAP and TAZ cooperatively regulate MCM2 – 7 expression in HCC cells after siRNA-mediated silencing. Administration of the YAP/TAZ inhibitor Verteporfin in human cancer cell lines reduced the expression of all MCMs in HCC cells. Vice versa, the inducible overexpression of YAP S127A in transgenic mice showed significantly increased levels of MCM2 – 7 associated with signs of pathological mitosis. In vitro analyses revealed that transcription factors of the TEAD family (TEAD 1 and TEAD 4) were the most relevant YAP binding partners involved in the regulation of MCMs. Using ChIP analysis, we exemplarily showed that YAP and TEAD 4 directly bound the MCM2 promoter. Expression data derived from 242 HCC patients

illustrated an association between YAP and MCM mRNA levels, with MCMs significantly correlating with worse overall survival and early cancer recurrence [3]. Furthermore, immunohistochemical stains of tissue micro-arrays revealed a significant correlation between YAP overexpression and nuclear MCM2 enrichment in human HCC tissues. In summary, our results suggest that the Hippo pathway effectors YAP and TAZ support HCC proliferation through the direct and coordinated induction of MCM family members. Thus, combined inhibition of YAP and TAZ (e.g. by Verteporfin) or perturbation of MCM activity (e.g. Ciprofloxacin) might represent efficient therapeutic approaches for the treatment of HCC patients with YAP/TAZ overexpression.

4.35 p53 transcription factors control apoptosis susceptibility by regulation of Mcl1

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Background: Hepatocellular carcinoma (HCC) represents a complication of liver cirrhosis limiting the curing option by liver transplantation. Depending on their splice variants – with transactivation (TA) domain or dominant negative (DN) – p53-family transcription factors (p53, p63, p73) exert tumorsuppressive or oncogenic functions in cell cycle by transcriptional regulation of a specific set of genes. In previous studies we identified the MCL1 gene (Myeloid cell leukemia sequence 1, Mcl-1) as potential target gene of the p53 family and confirmed its prognostic relevance in HCC. Mcl-1 is a member of the Bcl-2 protein family being involved in the control of mitochondrial integrity. Mcl-1 represents an anti-apoptotic member of the Bcl-2 family, supporting cell survival by binding and inhibition of pro-apoptotic Bcl-2 proteins. Aim of this study was to elucidate the impact of p53 family members on MCL1 gene regulation. **Methods:** Hep3B cells were transfected with rAd-GFP, -p53, -TAp63α, -TAp73β, -DNp63α, and DNp73β. MCL1 expression was measured by real time qPCR. Western Blot analyses determined intracellular levels of Mcl-1 after specific siRNA interference. Potential binding sites of p53 family members in the MCL1 locus were identified by database analyses (pDraw32, Husar, MatchTM) and verified by luciferase reporter assays. Direct protein interactions of Mcl-1 and p53 proteins were evaluated by LUminescence-based Mammalian IntERactome mapping (LUMIER) technology. **Results:** p53 and the isoforms TAp63 and TAp73 inhibited MCL1 expression, whereas ΔNp63α and ΔNp73β induced transcription of the MCL1 gene. These effects were confirmed for Mcl-1 protein levels. Reduction of Mcl-1 protein was abrogated after silencing of p53, TAp63 and TAp73. Database analyses identified two potential p53 binding sites and one potential p63 binding site each in promoter, intron 1 and 2 of the MCL1 gene. Luciferase reporter assays of these cloned putative binding sites confirmed a regulation of the MCL1 gene by p53 family members. Furthermore, a direct protein-protein interaction of Mcl-1 with p53 and TAp63, respectively, was detected. **Conclusion:** p53 family members directly regulate the expression of the MCL1 gene. p53, TAp63 and TAp73 are potent repressors, whereas DNp63 and DNp73 act as inducers of MCL1. On posttranslational level, Mcl-1 interacts with p53 and TAp63. Since Mcl-1 has anti-apoptotic functions, a downregulation of Mcl-1 by TA-isoforms of p53 proteins results in an increase of apoptosis susceptibility. HCC is often associated with chemotherapeutic resistance, which could be overcome by reconstitution with p53, TAp63 and TAp73 or a direct inhibition of Mcl1.

4.36 p73 is a potent inducer of IGFBP4 gene expression in hepatocellular carcinoma

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Background: p53-family members p53, p63 and p73 are known for their involvement in regulating cell cycle, cell senescence and apoptosis. In their role as transcription factors and depending on their splice variants – with transactivation domain (TA) or dominant negative (DN) – p53 and its siblings activate or inhibit the transcription of specific target genes. We previously identified the gene for Insulin-like Growth Factor Protein 4 (IGFBP4) as potential p53-family target gene with prognostic relevance in hepatocellular carcinoma (HCC). In contrast to p53, the IGF system takes part in tissue growth and cell survival. IGFBP4 acts as inhibitor limiting IGF effects suggesting a possible interaction with p53 affairs. Considering this feature, we aimed to characterize the regulatory influence of p53 family members on IGFBP4. **Methods:** Hep3B cells were transfected with rAd-p53, -TAp63, -TAp73, -DNp63, and -DNp73. Transcriptional regulation of IGFBP4 was determined by real time qPCR. Intra- and extracellular IGFBP4 protein levels were examined by Western Blotting and ELISA. TRANSFAC database analysis was performed to identify potential p53-family binding sites in the IGFBP4 locus. Identified sequences were cloned, deleted and analyzed in luciferase reporter assays to evaluate binding of p53-family members. **Results:** IGFBP4 expression was increased by more than 30-fold in TAp73-transfected Hep3B cells, by more than 15-fold in DNp63- and by 3-fold in p53-transfected cells. Induction of intracellular IGFBP4 protein was detected in all transfected Hep3B cells, whereas extracellular IGFBP4 levels were only measurable after TAp73 and DNp63 transfection. Database analysis identified 2 putative p73 binding sites within intron 1 of the IGFBP4 gene. Intron 1-dependent luciferase activity was increased by up to 20-fold in TAp73-transfected cells. This induction was reduced by up to 70% when one of the putative binding sites was deleted. **Conclusion:** These results identify the IGF inhibitor IGFBP4 as novel target gene for TAp73 in HCC. By demonstrating for the first time the interaction of TAp73 and IGFBP4 we enhance our knowledge in a so far unknown association of p53-family network and IGF signaling. Since in an independent study we identified IGFBP2 as an additional p53-family target gene, these results highlight the link between p53-family-mediated tumor-inhibiting mechanisms and IGF-dependent cell proliferation. We therefore suppose that the particular balance of these pathways decides on growth, cancerogenesis and treatment response.

4.37 Percutaneous ultrasound-guided cyst sclerotherapy in patients with polycystic liver disease

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Background: Polycystic liver disease (PCLD) is a genetic disease causing hepatomegaly and local cystic complications. To date, there is no established medical therapy for PCLD. We present our results of percutaneous minimal-invasive cyst sclerotherapy in patients with PCLD. **Patients and methods:** 301 pat. with PCLD were followed by our centre between 2001 – 2008 and retrospectively analysed. Mean age was 50 ± 11 years with 19% male. Additional polycystic kidney disease was found in 63% of pat. All pat. received a special questionnaire to report their subjective follow-up. Follow-up was 33 ± 27 months. 31 pat. underwent liver transplantation during this period. A total of 31 pat. died during follow-up, 9 of them with liver-associated cause of death. Cyst sclerotherapy was performed in pat. with a minimum cyst size of 8 – 10 cm or with symptomatic cysts. For sclerotherapy, cyst fluid was aspired with a 20G needle under ultrasound-guidance. Around 10% of the aspired volume was then administered into the cyst in form of 1% ethoxysclerol. In cysts with a diameter of > 15 cm, cyst drainage was performed to empty them first and perform sclerotherapy via drainage thereafter. In infected or sanguinous cysts, they were rinsed with NaCl 0,9% instead of sclerotherapy. **Results:** A total of 422 cyst therapy sessions of 903 cysts with a mean diameter of 8,1 ± 3,7 cm was performed in 149 pat. (50%). This included 705 sclerotherapies, 33 drainages and 110 cyst rinsing therapies. Indications for cyst therapy were especially symptomatic cysts (67%) and cyst size (48%). The questionnaire of 150 pat. could be analysed, 85 of them underwent cyst therapies leading to a subjective improvement of symptoms in 88% of them. Complications of cyst therapy were noted in 26% of cases, mostly local pain (16%) or inflammatory signs (16%). Bleedings occurred in < 1%. **Conclusions:** Percutaneous ultrasound-guided cyst sclerotherapy is easy, effective, cheap and with few complications to improve symptoms of patients with PCLD.

4.38 Role of Fbxw5 in the induction of centrosome abnormalities and liver tumor formation

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Centrosome duplication is tightly regulated process and should occur only once per cell division. Furthermore, genomic stability requires that centrosomes and chromosomes are segregated equally to the cell poles during mitosis. However in most types of tumors the centrosome duplication cycle is corrupted and it is unclear whether centrosome abnormalities are cause or consequence of the tumor formation process. Liver tumors belongs to the most severe cancer types, moreover frequently displays centrosome amplification [1]. Previous work in our group indicates a central role for the ubiquitin E3 ligase SCFFBXW5 in the initiation of the centrosome cycle by regulating the level of SASS6. RNAi mediated destabilization of Fbxw5 induces centrosome amplification and the formation of multinucleated cells [2]. Based on these findings, we decided to investigate the role of Fbxw5 in the induction of centrosome abnormalities in the liver cell cycle and in liver tumor formation. To this end, we take advantage of well-established shRNA technology as well as CRISPR/Cas9 to silence mouse Fbxw5, either via retroviral transfer in vitro or through a transposon based system in vivo using sleeping beauty transposase. We show that knockout of Fbxw5 leads to impaired proliferation and migration in vitro. Moreover after suppression of Fbxw5 cells display multipolar spindles accompanied by a delay in the transition from M to G1 phase. Although these multipolar spindles have their origin in amplified centrosomes, which may result in genomic instability, our in vivo studies reveal that the abnormality in centrosome number is not sufficient for tumor formation in wild type, p19-/- and p53-/- backgrounds. Thus, we plan to perform screening using Fbxw5 deficient cells and genome wide CRISPR/Cas9 library to identify further players in centrosome duplication. **References:** [1] Gönczy, P., Centrosomes and cancer: revisiting a long-standing relationship. Nature Reviews Cancer 15, 639 – 652 (2015) [2] Puklowski, A., et al., The SCF-FBXW5 E3-ubiquitin ligase is regulated by PLK4 and targets HsSAS-6 to control centrosome duplication. Nat Cell Biol, 2011. 13(8): p. 1004 – 9.

4.39 Role of the IQGAP1/IQGAP2 imbalance in Liver Cancer

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Background and Aims: The scaffold proteins IQGAP1 and IQGAP2 are involved in the regulation of several cellular processes affecting liver homeostasis. Several studies associated specific cellular functions to the localization and/or the protein levels of IQGAP1 and IQGAP2, which seem to modulate pathways related to liver cancer (e.g. Hippo-YAP, ß-catenin, MAPK). While IQGAP2 is exclusively expressed in the liver, the IQGAP1 protein is ubiquitously expressed, albeit at low levels in healthy adult liver. Recently, a switch in the IQGAP1 and IQGAP2 expression during the progression of liver cancer has been shown, which results in increased IQGAP1 levels and strongly reduced or even abolished IQGAP2 expression in human hepatocellular carcinoma (HCC). **Methods:** We took advantage of different HCC-derived cell lines ubiquitously presenting high IQGAP1 expression but varying levels of IQGAP2 (Huh6 and Huh7 high levels, HLE and SK-Hep1 low levels). Expression levels of IQGAP1, IQGAP2 and candidate downstream targets (YAP, ß-catenin, AKT) together with potential common effectors (CDC42, angiomotin (AMOT)) were measured by Western immunoblot and quantitative PCR, respectively. Co-immunprecipitation (Co-IP) experiments were carried out to identify interaction partners of IQGAP1 and IQGAP2. Affinity pull-down assay was used to detect the CDC42 activity in HCC cell lines. As bile acids may affect the IQGAP1 expression level [Cell Rep. 2013 Nov 27;5 (4):1060 – 9], HCC cells were treated with chenodeoxycholic acid (CDCA), and the effect on IQGAP1/2 complex composition and CDC42 activity was determined. **Results:** Western immunoblot analysis revealed that HCC cells with high IQGAP2 expression (Huh6, Huh7) show high ß-catenin levels, while HCC cells characterized by a high IQGAP1 expression reveal an upregulation of YAP expression. Co-IP analyses revealed that IQGAP1 interacts with YAP and AMOT in a possible functional complex in HLE and Huh6 cells, while IQGAP2 exclusively binds to AMOT in Huh6 cells. Furthermore a binding between IQGAP1 and CDC42 was shown for all HCC cell lines analyzed, whereas the interaction between CDC42 and IQGAP2 could be only demonstrated in the IQGAP2-high expressing cell lines Huh6 and Huh7, respectively. Low dose CDCA (50 µM) treatment increased the binding of IQGAP2 to CDC42 in Huh6 and Huh7 with no interaction detectable in HLE cells. **Conclusion and Outlook:** This study demonstrated the relevance of the IQGAP1/IQGAP2 ratio for the modulation of downstream signaling pathways involved in liver cancer progression (e.g., YAP, ß-catenin, CDC42). HCC cell lines with a high IQGAP2/IQGAP1 ratio (Huh6, Huh7) showed a higher affinity binding between CDC42 and IQGAP2. Further in vitro and in vivo experiments are needed to understand the dynamic interplay between CDC42 and IQGAP1 respectively IQGAP2, which seem to modulate specific cellular functions and mechanisms critical for the selection of cancer cells during liver carcinogenesis.

4.40 Room-temperature susceptometry allows the sensitive and non-invasive assessment of liver iron

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Objectives: Liver iron not only accumulates in hemochromatosis but also in various chronic liver diseases such as HCV and ALD where it has been identified as an important prognostic and cancerogenic factor. Unfortunately, the non-invasive and cost-efficient assessment of liver iron is still insufficiently resolved. We here establish room temperature susceptometry (RTS) in a large and heterogeneous cohort of patients with iron and liver disorders. **Methods:** 261 patients with or without signs of iron overload or liver disease were prospectively enrolled. Magnetic susceptibility (MS) was determined using RTS (Insight magnetics, San Diego). 32 patients also underwent liver biopsy with histological determination of fibrosis stage (Chevalier Score, Kleiner Score) and the semiquantitative degree (0 – 4) of iron (Prussian Blue staining). In 30 patients, liver iron concentration (LIC) was also determined by atomic absorption spectroscopy (AAS). In addition, in vitro studies were performed. **Results:** In vitro studies showed that MS linearly ($r2 = 0.998$) depends on iron concentration over a huge range with a detection limit of 15.9 µg iron per g ww. We next studied the influence of air and overlaying fat as the most relevant confounders in humans on MS to accurately detect LIC by RTS. An optimized algorithm that considered skin-to-liver capsule distance showed 85% valid measurements in a clinical cohort with a mean BMI of 25.1 ± 6.3. MS correlated highly significant with AAS-measured LIC ($r = 0.77$; $p < 0.01$). Notably, correlation of MS with LIC by AAS was still significant when patients were not performing the exhaling maneuver but continued to breath normally (0.68; $p < 0.01$). Mean LIC was found to be 185 µg/g in controls, 291 µg/g in cirrhotics, and 355 or 1655 µg/g in patients with mild or severe iron overload, respectively. In one patient with newly diagnosed HFE-related hereditary hemochromatosis, RTS allowed sequential monitoring of iron depletion following phlebotomy over 12 months. **Conclusion:** RTS is a novel, noninvasive method to determine LIC. It could be a cost-effective method for future liver iron-screening and follow-up studies e.g. in response to iron chelation therapy.

4.41 Serum Cell Death Biomarker Mirrors Liver Cancer Regression after Transarterial Chemoembolisation

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Hepatocellular carcinoma (HCC) represents an increasing health problem with limited therapeutic options. In patients with intermediate disease stage, transarterial chemoembolization (TACE) is widely applied. Treatment response is routinely assessed by imaging techniques according to the international response evaluation criteria in solid tumors, which consider tumor regression (RECIST) or additionally tumor necrosis (mRECIST). Evaluation of treatment response, however, by these methods is time- and cost-intensive and usually performed at earliest several months following TACE. We therefore investigated the suitability of novel non-invasive cell death biomarkers for an earlier prediction of TACE re-

sponse. For this purpose we analyzed activation of pro-apoptotic caspases and the proteolytic cleavage of the caspase substrate CK-18 in liver tissues and sera from HCC patients by immunohistochemistry, a luminometric substrate assay and ELISA. Both caspase activity and caspase-cleaved CK-18 fragments were elevated in HCC patients compared to healthy controls. CK-18 serum levels significantly increased during the first three days and peaked at day two following TACE. Interestingly, we found significant differences in CK-18 levels between patients with and without tumor regression. Detection of CK-18 fragments revealed a promising performance for the early prediction of TACE response with an area under the curve value of 0.76. In conclusion, caspase-cleaved CK-18 levels mirror liver cancer regression and allow an earlier prediction of TACE response. The concordance with mRECIST suggests that detection of CK-18 levels immediately after TACE might be used as short-term decision guide to continue or change HCC therapy.

4.42 Short term effect of transarterial chemoembolization (TACE) on microsomal liver function by means of 13C-methacetin breath test (MBT) in patients with hepatocellular carcinoma (HCC)

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Background: Chemoembolization is a frequently applied therapeutic modality for un-resectable HCC, and is suggested as first line-therapy for patients at intermediate disease stages. Transcatheter intraarterial injection of cytotoxic agents and embolization of tumor feeding arteries induce a strong cytotoxic and ischemic tissue effect potentially leading to hepatic dysfunction. The MBT is a feasible non-invasive function test for the assessment of hepatic functional reserve, overall prognosis and complications in patients with chronic liver diseases. (Goetze et al., APT 2007, AASLD 2015; Stravitz et al. J Hepatol 2015). **Aims:** To assess in a pilot study prospectively the short term effect of conventional TACE on hepatic functional reserve by MBT and on static functional and inflammatory parameters. **Methods:** 14 male patients with liver cirrhosis of different etiologies and unresectable HCC (all BCLC B and Child Pugh class A, age 65.3 ± 3.1 y., BMI 27.9 ± 1.2 kg/m2, MELD 10.2 ± 0.8, all mean ± SEM) were studied. Each patient received 75 mg of 13C-methacetin dissolved in 100 ml of water before (d0), 24 h (d1) and 72 h (d3) after conventional TACE therapy. The 13C/12C ratio in breath was determined over 60 minutes in 10 minute intervals by nondispersive isotope selective infrared spectroscopy (IRIS, Kibion, Sweden) as delta over baseline (DOB [‰]) and was expressed as maximal 13C/12C ratio (DOBmax [‰]) and calculated percentage dose rate (PDRmax [%/h]). In addition, static liver function and inflammatory markers were assessed on each study day. Data was analyzed by one linear mixed effects model fit and by linear regression analysis (R 3.2.3). **Results:** Transarterial chemoembolization induced a sustained decrease in microsomal liver function 24 h and 72 h following therapy [d0 (DOBmax, PDRmax): 12.2 ± 1.8‰, 15.7 ± 3.2%/h; d1: 8.8 ± 1.6‰, 10.8 ± 2.6%/h; d3: 9.5 ± 1.7‰, 11.0 ± 4.6%/h; p = 0.04 and 0.07 d0 vs. d1; p = 0.13 and 0.08 d0 vs. d3], which was most pronounced in HCC patients with a PDRmax> 5%/h (n = 8) and lower MELD values (9.3 vs. 11.5) at d0 [d0 (DOBmax, PDRmax): 17.0 ± 2.5‰, 25.7 ± 3.7%/h; d1: 10.7 ± 2.5‰, 16.4 ± 4.0%/h; d3: 11.7‰, 16.8 ± 4.0%/h; p = 0.03 and 0.02 d0 vs. d1 and p = 0.05, p = 0.04 vs. d3]. Static liver function test remained unchanged [MELD d1: 10.1 ± 0.4; d3: 10.7 ± 0.4; p = 0.2 – 0.7 d0 vs. d1, d3] and inflammatory markers increased [WBC (103/µl), PCT (ng/ml), CRP (mg/dl): d0: 5.9 ± 0.9; 0.13 ± 0.03; 0.6 ± 1.3; d1: 8.0 ± 0.9; 0.22 ± 0.04; 1.2 ± 1.7; d3: 8.8 ± 0.9; 0.28 ± 0.05; 7.7 ± 1.8; p = 0.02, p = 0.04, p = 0.7 d0 vs. d1; p = 0.003, p = 0.005, p < 0.001 d0 vs. d3]. The increase of inflammatory markers were not associated with the decrease in liver function (data not shown). **Conclusions:** In contrast to previous reports using 13C-aminopyrine (Schuette et al. Z Gastroenterol 2015) in this pilot study a reduction in functional hepatic reserve induced by TACE is reflected by a sustained decrease in 13C-methacetin metabolism over 72 h whereas static liver function test remained unchanged. The postprocedural inflammatory response was not associated with the observed decrease in liver function. The MBT might therefore be helpful in the quantification of postprocedural liver function and prediction of decompensation, which will be addressed in a larger prospective clinical trial.

4.43 The effect of Transforming Growth Factor Beta family members on tumor initiating cells in primary liver cancer

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Aims: TGF-β is a major signaling pathway of the liver with pleiotropic effects on different processes and cell types. During liver cancer development TGF-β exerts tumor suppressor effects on normal hepatocytes and early carcinomas. However, in progressed tumor stages, the cytostatic effects of TGF-β are often lost due to disruption of the signaling pathway by disturbed expression of TGF-β signaling modulators, genetic and/or epigenetic alterations. This progressed stage is characterized as a "late TGF-β signature" which promotes the phenotypic switch from tumor suppressor to promoter in liver cancer, and confers to invasive and prometastatic properties. Besides this key role for HCC development and progression the effect of TGF-β on stem-like cancer cells are not well defined. **Methods:** Different HCC and CCA cell lines were exposed to different concentrations of TGF-β1 and TGF-β2 (1 ng/ml and 5 ng/ml). The effect of TGF-β on the stemness potential was studied by employing various established functional assays following a three day treatment. Further, stem cell markers were evaluated using qRT-PCR and flow cytometry. **Results:** Treatment of primary liver cancer cell lines with TGF-β1 and TGF-β2 led to a significant reduction in colony and spheroid forming ability in all cell lines. The number of putative stem-like cells was also diminished reflected by the frequency of the side population and down-regulation of stemness markers CD133, EpCAM and ABCG2. Further, a higher expression of CD44 was observed after exposure to TGF-β1 accompanied by activation of epithelial-mesenchymal-transition (EMT) transition markers, SNAIL and down regulation of the cell-cell contact protein E-Cadherin after stimulation with TGF-β1. Consequently, enhanced migratory and invasive properties were observed after the stimulation. **Conclusions:** In conclusion, we here confirm the cytostatic effect of TGF-β1 and TGF-β2 by reducing the frequency of stem-like cancer cells in both HCC and iCCA. On the other hand, TGF-β1 presents as a regulator of EMT and invasive properties in progressed PLCs. These context-dependent dichotomic effects should be considered in TGF-β based therapeutic approaches.

4.44 The lack of the organic cation transporter OCT1 at the plasma membrane of tumor cells precludes a positive response to sorafenib in patients with hepatocellular carcinoma

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Background: Sorafenib is the drug of choice in the treatment of advanced hepatocellular carcinoma (HCC), however its beneficial effect is limited by the effectiveness of mechanisms of chemoresistance present in tumor cells, which include downregulation and/or impaired function of plasma membrane transporters accounting for drug uptake. Since the organic cation transporter 1 (OCT1) plays a major role in sorafenib uptake and OCT1 expression is decreased in HCC, which has been associated with poorer response to sorafenib, here we have elucidated whether the presence of the protein at the plasma membrane rather than the mRNA/protein levels of the transporter was related to the outcome of the pharmacological treatment. **Methods:** The multicenter retrospective study involved liver tumor biopsy in 39 patients with sorafenib therapy for advanced HCC with known outcome (survival, radiological response) of a minimum duration of 4 weeks, collected at three German hospitals (TRANSFER study). Endpoint was the relationship between clinicopathological features and the result of immunohistological examination. Immunostaining was performed on whole sections from paraffin-em-

bedded material using primary anti-OCT1 antibody. Slides were reviewed independently by two observers blinded to clinical data. Tumors were classified according to a simplified staining score as absent, weak, moderate or strong, and taking into account the localization of the staining at the plasma membrane as positive or negative. **Results:** The results confirmed OCT1 downregulation in approximately half of the cases investigated (10.3% absent, 38.4% weak). However, only one third of the tumors expressing OCT1 displayed plasma membrane location of the protein (15.4% vs. 35.9% cytosolic expression). When comparing HCC with and without OCT1 expression, no different response to sorafenib was found. When tumors expressing OCT1 at the plasma membrane were considered separately, a marked longer survival was found in these cases (Log Rank $p < 0.001$). No association between OCT1 expression at the plasma membrane with the stage of the tumor, the previous treatment with TACE or the radiological response was seen. In conclusion, these results indicate that the presence at the plasma membrane, rather than the overall OCT1 expression, is related with better outcome of HCC patients treated with sorafenib. A prospective study is warranted to investigate the use of OCT1 immunostaining for the guidance of systemic HCC treatment.

4.45 Tumor suppressor genes SORBS3 and SH2D4A collaborate to repress IL-6/STAT3 signaling in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the most common type of liver cancer, which is the second-leading cause of cancer-related mortality worldwide. Chronic infection with hepatitis B (HBV) and C viruses (HCV) and other inflammatory liver diseases such as alcoholic and non-alcoholic steatohepatitis contribute to the development of HCC, making HCC a paradigm for inflammation and virus-induced cancer. In patients with chronic liver disease interleukin-6 (IL-6) serum levels are elevated and increase even more when HCC develops. But the regulatory mechanisms of IL-6 signaling in hepatocarcinogenesis are still poorly understood. Recently, we demonstrated that loss of chromosome 8 p tumor suppressor genes SH2D4A and SORBS3 is associated with poor prognosis. Furthermore, increased IL-6 signaling could be observed in patients with chromosome 8 p gene signature loss. The goal of this study was to dissect the molecular mechanisms of SORBS3 and SH2D4A mediated tumor suppression related to IL-6 signaling. We found that SORBS3 and SH2D4A function in a convergent manner to inhibit IL-6 signaling. Overexpression of SORBS3 or SH2D4A in HCC cell lines led to significant reduction of cell proliferation and colony formation. This effect was even further increased when both genes were co-expressed. SORBS3 and SH2D4A each decreased IL-6 target gene expression and reduced IL-6 induced STAT3 transcriptional activity in a luciferase reporter assay. SH2D4A physically interacts with STAT3 in in vitro co-immunoprecipitation and in situ proximity ligation assays (PLA). Thereby, SH2D4A blocks STAT3-dimerisation and leads to retention of STAT3 in the cytoplasm. SORBS3 directly binds and co-activates Estrogen Receptor alpha (ERα) leading to repression of STAT3 signaling. Furthermore, we observed binding of SH2D4A and SORBS3 to STAT1. However, STAT1 could not be activated by IL-6 in HCC cell lines. Thus, it appears that IL-6-mediated signaling is specific to STAT3 in HCC. Applying a tissue microarray ($N = 127$) of human HCC tissues, we found that loss of SH2D4A expression is associated with decreased infiltration of cytotoxic and regulatory T cell populations suggesting loss of tumor immune surveillance. Thus, loss of chromosome 8 p tumor suppressor genes SORBS3 and SH2D4A leads to increased STAT3/IL-6 signaling and decreased infiltration of cytotoxic and regulatory T cells. Therefore, SORBS3 and SH2D4A functionally cooperate to inhibit STAT3-mediated IL-6 signaling in HCC.

4.46 Tumor suppressor microRNA-198 is actively transported out of liver cancer cells

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Background and Aim: microRNA-198(miR-198) has been proven as a tumor suppressor in liver cancer cells, inhibiting cell growth and proliferation. Previous studies have shown that miR-198 is the most downregulated miRNA during liver cancer progression. Therefore, we aimed to study the mechanism of miR-198 decrease in liver cancer cells. **Methods:** A miR-198 expression cassette was cloned downstream to a tet-on promoter and then stably transfected into huh7 liver cancer cells. Isolated total RNA was applied to real time PCR miR-198 quantification. Furthermore, vesicles were isolated from cell culture supernatants by serial centrifugation and the vesicular proteins and RNA were characterized by proteomics, immunoblot and real time PCR. MTT cell growth tests and migration assays were performed in order to study the function of miR-198 overexpression. **Results:** In contrast to treatment of liver cancer cells with miR-198 mimics, miR-198 overexpression induces no cell growth inhibition. However, it leads to an immense miR-198 release from cancer huh7 cells, which is associated with release of vesicles into the cell supernatants. Characterization of vesicles proved the exosome markers, CD63 and HSP70. Importantly, we show that exosomes, released from liver cancer cells and carrying miR-198, can be up-taken by other cell types. Furthermore, cells, without endogenous miR-198 expression, respond with growth inhibition after exosome treatment. **Conclusion:** In liver cancer cells, tumor suppressor miR-198 is tightly controlled and is preferably sequestered in vesicles and transported into medium via exosomes. This altogether can provide new insights in exosomal miRNA release and intercellular communication pathways.

4.47 Tumorsuppressive MicroRNA-188-5p Reveals Novel Oncogenes for Hepatocellular Carcinoma

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Background & Aims: MicroRNAs are known to be dysregulated in Hepatocellular Carcinoma (HCC) and to play crucial roles in tumor development and progression. In a preliminary study, our group found that miR-188-5p is downregulated in activated synovial fibroblasts in rheumatoid arthritis (RASF). RASF reveal tumor-like features such as enhanced migration and proliferation. Re-Expression of miR-188-5p strongly inhibited migration in RASF (Int J Clin Exp Pathol, 2015). Chloride voltage-gated channel 5 (CLCN5), which is the host gene for miR-188-5p, was shown to be induced by Hepatocyte nuclear factor (HNF) 1 (Am J Physiol Renal Physiol., 2010). Interestingly, our group demonstrated in a previous study that HNF-1 is a lost tumorsuppressor in HCC (Gut, 2008). Therefore, in this project, our aim was to explore a possible role of miR-188-5p in cancer development and progression with a focus on HCC. **Methods & Results:** For functional analysis, transient transfection of pre-miR-188-5p was performed in HCC cell lines (PLC, Hep3B, and HepG2). MiR-188-5p markedly inhibited migration and proliferation and reduced clonogenicity in HCC cell lines. Quantitative RT-PCR analysis showed that miR-188-5p expression was significantly downregulated in HCC tissue samples from patients as compared to corresponding non-tumorous liver tissues. Moreover, miR-188-5p expression was strongly reduced in HCC cell lines as compared to primary human hepatocytes (PHH). Furthermore, first experiments showed that HNF-1 overexpression enhances miR-188-5p expression in HCC cell lines, and HNF-1 induced inhibition of clonogenicity was 'rescued' by anti-miR-188-5p. For determination of potential novel target genes of the miR-188-5p in HCC, cDNA array analysis comparing HCC cells with and without re-expressed miR-188-5p was performed, and revealed a list of several potential novel target genes for miR-188-5p, including Ephrin B2 (EFNB2), Discs large MAGUK scaffold protein 5 (DLG5), and Tumor protein p63 regulated 1 like (TPRG1L). We will further investigate these potential novel oncogenes in HCC development and progression and their regulation by miR-188-5p. **Conclusions:** We found that a mostly unknown microRNA in cancer, miR-188-5p, is downregulated in HCC cells and re-expression experiments revealed potent tumorsuppressive functions. Moreover, we found that miR-188-5p expression is regulated by Hepatocyte Nuclear Factor 1, a downregulated tumorsuppressor in HCC. cDNA array analysis comparing

HCC cells with and without re-expressed miR-188-5p revealed a list of several potential novel target genes for miR-188-5p. We will further investigate these potential novel oncogenes and evaluate possible therapeutic approaches for HCC. **References:** [1] Hellerbrand C, Amann T, Schlegel J, et al. The novel gene MIA2 acts as a tumour suppressor in hepatocellular carcinoma. Gut (2008) [2] Ruedel A*, Dietrich P*, Schubert T, et al. Expression and function of microRNA-188 – 5 p in activated rheumatoid arthritis synovial fibroblasts. Int J Clin Exp Pathol (2015) (*contributed equally) [3] Tanaka K, Terryn S, Geffers L, et al. The transcription factor HNF1α regulates expression of chloride-proton exchanger ClC-5 in the renal proximal tubule. Am J Physiol Renal Physiol. (2010)

5. Virus Immunology

5.1 A TCF1 expressing memory-like population of Hepatitis C virus-specific CD8+ T cells is maintained after DAA-mediated viral elimination

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Chronic Hepatitis C virus (HCV) infection results in impaired HCV-specific CD8+ T-cell responses, a phenomenon called T-cell exhaustion. Exhausted CD8+ T cells exhibit reduced cytokine production and proliferative capacity, co-express inhibitory molecules (e.g. PD-1) and lack memory markers like IL-7R a-chain (CD127) or transcription factor TCF1. The mechanisms responsible for CD8+ T-cell exhaustion are not completely understood, however, one key feature seems to be the prolonged and continuous exposure to antigen. In chronic HCV infections, an unanswered question of clinical importance is the fate of exhausted HCV-specific CD8+ T cells after viral elimination. By taking advantage of the recently approved direct acting antivirals (DAA) in HCV therapy we were able to address this issue for the first time in a highly relevant clinical setting. Here, we analyzed phenotype and function of HCV-specific CD8+ T cells prior, during and after successful DAA therapy in a cohort of 29 patients by using a novel peptide/MHCI tetramer enrichment strategy. Our results can be summarized as follows: First, we could identify different subsets of HCV-specific CD8+ T cells co-existing during antigen persistence. We found a CD127-PD1hi subset that exhibited a profile of terminal exhaustion and a CD127+PD1+ subset that was less differentiated including protection from apoptosis by high expression of anti-apoptotic molecule Bcl2. Second, we could assign memory-like characteristics to the CD127+PD1+ subset of HCV-specific CD8+ T cells including long-term antigen-independent survival after DAA-mediated antigen removal. Of note, we were able to monitor the HCV-specific CD8+ T-cell response in a case of viral relapse. In this patient, re-exposure to HCV led to vigorous expansion of HCV-specific CD8+ T cells and generation of terminally exhausted CD127-PD1hi cells suggesting a progenitor-progeny relationship within a heterogeneous HCV -specific CD8+ T-cell population. Third, these memory-like CD127+PD1+ HCV-specific CD8+ T cells share phenotypic, molecular and functional properties of both T-cell memory and T-cell exhaustion clearly demonstrating divergent T-cell differentiation in chronic compared to spontaneously resolved HCV infection. Finally, on a molecular level CD127+PD1+ HCV-specific CD8+ T cells were defined by expression of TCF1 that was linked to the proliferative potential of the overall HCV-specific CD8+ T-cell population. Thus, the CD127+PD1+ subset contained the proliferative capacity of HCV-specific CD8+ T cells during and after antigen persistence and appears to be central for the maintenance of HCV-specific CD8+ T-cell populations. In sum, our study reveals that memory potential of human virus-specific CD8+ T cells is not precluded by chronic infection even after years of chronic antigen exposure. These results have clear implications for protection from re-infection after antigen persistence and for future immunotherapeutic approaches.

5.2 Identification of a highly cross-reactive CD8+ T cell repertoire that recognizes an HEV peptide and an apoptotic epitope

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Introduction: Hepatitis E virus (HEV) infection is associated with extrahepatic manifestations, including autoimmune disorders. Several mechanisms have been suggested by which infections can initiate autoimmunity. One mechanism is cross-reactivity, where a foreign antigen shares sequence or structural similarity with self-antigens. Another mechanism could be that T cells are responsive to caspase-cleaved (apoptotic) antigens derived from effector T cells undergoing apoptosis, thereby contributing to immunopathology of autoimmune diseases. Here we hypothesize that CD8+ T cells can be cross-reactive to HEV antigens as well as to apoptotic antigens. **Objectives:** To elucidate cross-reactivity of CD8 + T cells to HEV and apoptotic epitopes. **Patients and Methods:** CD8+ T cells isolated from HEV seronegative, healthy individuals were expanded in-vitro using HEV genotype 3 overlapping peptides spanning all 3 open reading frame-encoded proteins. T cell response was examined by intracellular cytokine staining (ICS) while cross-reactivity was determined by the frequency of apoptotic epitope-specific CD8+ T cells as measured by dextramer staining. Cross-reactive CD8+ T cells were sorted and the T cell receptor (TCR) repertoire was analysed. **Results:** Notable HEV-specific CD8+ T cell were detected in 3 out of 4 unexposed individuals tested by ICS. In one donor, we detected strong cross-reactivity between an HEV 9mer peptide and apoptotic epitope MYH9 – 478. After 14 days in-vitro stimulation with the HEV peptide, up to 25% of CD8+ T cells were MYH9 – 478 dextramer positive, which produced cytokine upon HEV peptide restimulation. Interestingly, sequence analysis of TCR showed a highly oligoclonal repertoire, with a maximum of two T cell clonotypes. All clones showed an unusual CDR3 motif in the TCR β chain with multiple glycines, which may help to explain the flexibility of the TCR to interact with two very different epitopes (2 out of 9 a.a homology). **Conclusion:** Our study provides evidence for cross-reactive HEV-specific CD8+ T cells in HEV seronegative individuals. The cross-reactivity with apoptotic epitopes gives these T cells autoinflammatory potential. The unusual glycine-rich region in TCR may be a feature of highly cross-reactive T cells and of diagnostic characteristic.

5.3 Ignorance of hepatic autoantigen in thymus and periphery enables the development of autoimmune liver disease

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Background/Aims: The liver has a distinct capacity to induce immune tolerance, which seems to be actively maintained by various regulatory mechanisms including Tregs and inhibitory T cell stimulation. However under yet unclear conditions, hepatic tolerance can be broken, enabling the development of autoimmune liver disease. **Methods:** To learn how loss of hepatic tolerance might occur we have generated mice, which express an MHC class II-restricted immunodominant T cell epitope of the Lymphocytic Choriomeningitis Virus glycoprotein (GP61 – 80) specifically on hepatocytes. We constructed a mutated invariant chain (Ii) in which the CLIP peptide sequence was replaced by the GP61 – 80 peptide sequence, and inserted the mutated gene flanked by loxP sites into the ROSA26 gene, as has been done before with a similar construct (Frommer F et al. J Immunol 2008). Conditional expression in hepatocytes was achieved by breeding with Alb-Cre x Smarta mice, which were also transgenic for a T cell receptor recognizing the GP61 – 80 peptide. Alternatively, conditional expression was achieved in macrophages by breeding with LysM-Cre x Smarta mice. **Results:** Conditional expression of the GP61 – 80 peptide in macrophages resulted in deletion of autoreactive CD4 T cells in the thymus and virtual absence of antigen-specific CD4 T cells from the periphery. In contrast, conditional expression of GP61 – 80 in hepatocytes did not cause thymic deletion, resulting in abundance of autoreactive CD4 T cells in the periphery. Nonetheless, the majority of these cells were not activated in vivo and most mice did not develop autoimmune pathology. However at 20 – 30 weeks of age, about 25% of these mice spontaneously developed autoimmune liver inflammation, marked by elevated serum ALT levels (mean: 593 U/l versus 62 U/l in littermate controls; P = 0.031), periportal inflammatory infiltrates on liver histology as well as splenomegaly. **Conclusion:** Ignorance of liver

autoantigens bears a significant risk for accidental activation of autoreactive CD4 T cells. In the absence of active control mechanisms that effectively limit T cell activity, such accidental T cell activation can cause the development of autoimmune liver inflammation.

5.4 Analyse von Risikofaktoren einer CMV-Infektion bei lebertransplantierten Patienten sowie möglichen langfristigen Folgen

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Einleitung: Die Infektion mit dem Zytomegalievirus (CMV) stellt eine der häufigsten Komplikationen nach Lebertransplantation (LT) dar und kann in Folge der Immunsuppression zu schwerwiegenden Infektionen verschiedener Organsysteme führen. Trotz unterschiedlicher Untersuchungen gibt es bisher kein standardisiertes Vorgehen für Prophylaxe und Therapie von CMV-Infektionen nach LT. **Ziele:** In dieser Analyse sollen mögliche Risikofaktoren für die Entwicklung einer CMV-Infektion nach Lebertransplantation analysiert und Patientengruppen mit einem erhöhten Risiko herausgearbeitet werden. Zudem soll untersucht werden, welche langfristigen Konsequenzen eine CMV-Infektion für lebertransplantierte Patienten haben kann. **Methodik:** Die Daten von 892 lebertransplantierten und am hiesigen Zentrum betreuten Patienten wurden in einer Datenbank aufgenommen. Es erfolgte retrospektiv eine statistische Auswertung mittels Chi-Quadrat-Test, Fisher-Exact-Test und t-Test. Der CMV-Nachweis erfolgte mittels PCR. Ergebnis: Bei 192 der 833 Patienten (23,0%) wurde mindestens eine CMV-Infektion nach Lebertransplantation beobachtet, wobei wiederum 27,1% dieser Patienten mehrfache Infektionsepisoden zeigten. Der große Teil der Infektionen erfolgte im ersten Jahr nach Transplantation (69,9%). Die Serostatus-Konstellation von Donor (D) und Rezipient (R) stellt dabei einen wichtigen Risikofaktor dar, wobei das höchste Risiko bei den Konstellationen D+/R- (45,6%) und D+/R + (24,0%) bestand. Bzgl. der Grunderkrankungen der Patienten wurde beobachtet, dass Patienten mit einer primär sklerosierenden Cholangitis (PSC) signifikant häufiger eine CMV-Infektion erleiden (34,8%), während Patienten mit einer Hepatitis C Virus-Infektion signifikant seltener reaktivieren (14,7%). Auch bei Rauchern wurden signifikant seltener CMV-Infektionen beobachtet (12,8%). Ein höherer CRP-Wert zum Zeitpunkt der Transplantation war mit einem erhöhten Risiko einer CMV-Infektion assoziiert ($p = 0{,}016$). Patienten, bei denen eine CMV-Infektion beobachtet wurde, erlitten im Verlauf signifikant häufiger Cholangitiden ($p = 0{,}022$), und zwar unabhängig davon, ob es sich bei der Grunderkrankung um eine PSC handelt. **Schlussfolgerung:** Im Rahmen dieser Arbeit konnten verschiedene Risikofaktoren für das Auftreten einer CMV-Infektion nach LT erarbeitet werden. Dementsprechend ist davon auszugehen, dass bzgl. der Notwendigkeit einer Infektionsprophylaxe ein individualisiertes Vorgehen unter Berücksichtigung der jeweiligen Risikokonstellation sinnvoll ist.

5.5 Association of Toll-like receptor 4 (TLR4) gene polymorphisms with remission of chronic hepatitis B infections in female patients

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Background and Aims: Polymorphisms within the TLR4 gene are thought to influence the clinical course and outcome of HBV infections. They were shown to be associated with delayed progression of liver fibrosis and a reduced risk of development of hepatocellular carcinoma. We investigated the association of the occurrence of the TLR4 single nucleotide polymorphism (SNP) rs4986790 (A>G) and the polymorphism in 3'-untranslated region rs913930 (T>C) with spontaneous clearance of HBV infections. **Methods:** 519 patients with chronic hepatitis B (CHB group) and 169 subjects with spontaneous HBsAg clearance (SC group) were enrolled (595 Caucasians, 73 Asians and 20 Africans). In the CHB group, 26.4% (137/519) of the patients were HBeAg-positive and 47.6% (180/378) of the HBeAg-negative patients were inactive carriers. Genomic DNA was extracted from peripheral blood samples. The two SNPs were genotyped by polymerase chain reaction and melting curve analysis. Statistical analysis was made using Chi-square test and logistic regression analysis. **Results:** The genotype distributions of the two SNPs did not differed significantly between the CHB and SC groups. However, among female subjects, the presence of at least one minor allele was significantly more frequent in the SC group as compared to the CHB group (34.7% vs. 21%, $p = 0.015$), whereas this was not significantly different among male subjects. In the CHB group, the rs913930 CC genotype was significantly less frequent among the HBeAg positive patients as compared to the wild-type TT (15.7% vs. 31.1%, $p = 0.028$). In the regression analysis adjusted for age and gender, rs913930 CC was associated with a lower risk of HBeAg-positive HBV infection when compared to TT genotype (OR = 0.460, 95% CI = 0.226 – 0.936, $p = 0.032$). For the rs4986790 SNP, there was no association found with regard to HBeAg status. However, the presence of at least one minor allele of these two SNPs was significantly associated with HBeAg-negative status among female patients (OR = 0.406, 95% CI = 0.198 – 0.835, $p = 0.014$), but not among male patients. **Conclusions:** TLR4 polymorphisms at rs4986790 and rs913930 appear to be associated with remission of chronic HBV infections in females and could therefore have an impact on disease progression and outcome. Further studies are needed to confirm these results and elucidate the impact of these polymorphisms on the course and clinical outcome of HBV infection.

5.6 CEACAM1 induces B-cell survival and is essential for protective antiviral antibody production

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B cells are essential for antiviral immune defense because they produce neutralizing antibodies, present antigen, and maintain the lymphoid architecture. Here we show that intrinsic signaling of CEACAM1 is essential for generating efficient B-cell responses. Although CEACAM1 exerts limited influence on the proliferation of B cells, expression of CEACAM1 induces survival of proliferating B cells via the BTK/Syk/NF-κB-axis. The absence of this signaling cascade in naive Ceacam1-/- mice limits the survival of B cells. During systemic infection with cytopathic vesicular stomatitis virus, Ceacam1-/- mice can barely induce neutralizing antibody responses and die early after infection. We find, therefore, that CEACAM1 is a crucial regulator of B-cell survival, influencing B-cell numbers and protective antiviral antibody responses.

5.7 Characterization of T cell responses after stopping HBV therapy with nucleos(t)ide analogues (NA) in HBeAg-negative patients

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Introduction: In a prospective study we showed that stopping NA treatment in HBeAg-negative patients is safe and leads to a significant decline in HBsAg levels. Patients with high HBV-DNA and HBcrAg rebound showed the highest decrease in HBsAg levels at week 48. Interestingly, we could show that plasma cytokines/chemokine levels of IL-10, IL-12 and TNF were significantly increased early (4 weeks) and IP-10 at week 8 after treatment stop (presented at GASL 2016). This induction of cytokines could be an indicator for activation of immune responses. **Objec-**

tives: In order to understand the mechanisms leading to HBsAg decline, T cell responses were analysed before and after stop of NA therapy. **Patients & Methods:** In a prospective trial, 15 chronically HBeAg-negative patients stopped NA treatment. Multicolor flow cytometry was performed to characterize the ex-vivo immune cell phenotype at stop of treatment and 4, 8 and 12 weeks after stop. Additionally, HBV core-specific T cell responses were studied after in-vitro stimulation of PBMCs with core-specific overlapping peptides for 10 days and following restimulation. **Results:** HLA-DR was upregulated on T cells compared to healthy controls. Additionally, Ki-67 was increased on T cells at week 12 for those patients who had the strongest HBsAg decline. At stop of NA therapy, hardly any HBV-specific CD4+ and CD8+ IFNγ+ T cell responses were detectable. However, this changed in some, but not all patients after stop of treatment, when a virological relapse occurred. Overall increases of CD4+ T cell responses in 10/12 patients (SI> 2) and of CD8+ T cell responses in 9 out of 12 (SI> 2) were visible. In some patients, blocking PD-L1 could further augment this response. **Conclusion:** Stop of NA therapy and virological rebound led to changes in the peripheral blood T cell phenotype. HBV-specific T cell responses after stop of therapy could be induced after in vitro stimulation and the cells were more sensitive to in vitro checkpoint inhibition. The current data are relevant in relation to the discussion of new treatment concepts, i.e. combining NA treatment cessation with immunmodulatory therapies.

5.8 Clinical outcome and HBsAg variability of hepatitis B virus induced acute liver failure

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Introduction: Hepatitis B virus (HBV) infection remains a frequent cause of acute liver failure (ALF) worldwide. ALF can occur in 0.1 – 0.5% of infected patients and may lead to the urgent need of liver transplantation or death. The aim of this study was to scrutinize the clinical outcome of patients with HBV induced ALF and mutational patterns of HBV variants, which might contribute to ALF. **Patients and Methods:** Thirty four patients with HBV-induced ALF were hospitalized at the University Hospital, University Duisburg-Essen, from 2005 to 2016. Clinical, chemical and virological data from these patients was collected and the SHB region of the HBV genome detected at the time of ALF was sequenced per NGS. As a control group 80 patients with chronic hepatitis B (CHB) were included in this study. **Results:** Most HBV infections were caused by HBV genotype D (GT A: n = 6, GT B: n = 1, GT D: n = 23, GT E: n = 2 and GT F: n = 2). Antiviral therapy lead to the sustained suppression of HBV replication in all non transplanted patients and resulted in the loss of HBsAg and anti-HBs seroconversion (n = 13), the loss of HBsAg without anti-HBs seroconversion (n = 4) or chronic HBV (n = 1). Three patients died and four patients underwent liver transplantation despite antiviral treatment, while only one of them survived. Eight patients were lost to follow up. HBsAg mutations at positions and 31, 118, 127, 128 and 140 were significantly more frequent detected in HBV variants associated with ALF compared to chronic HBV ($p < 0.05$). **Conclusions:** Antiviral therapy prevented liver transplantation or death in most patients with HBV associated ALF. HBV variants associated with ALF did not carry a specific mutational pattern but some HBsAg mutations were significantly more often detected in HBV variants of patients with ALF.

5.9 Detection of a genetic footprint of the sofosbuvir resistance-associated substitution S282T after HCV treatment failure

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Substitutions associated with resistance against sofosbuvir (SOF) are rare even in patients after treatment-failure under a SOF-containing regiment. The major resistance-associated substitution (RAS) S282T in NS5B causes severe viral fitness costs and was associated with low viral loads in vivo. Accordingly, in the few patients where it was detected, the substitution rapidly reverted back to prototype in the absence of selection pressure. Here, we report a case of a GT3a infected patient with viral breakthrough under SOF/DCV therapy. At the time of breakthrough the RAS S282T was predominant in NS5B and then rapidly disappeared during follow-up by week 12 after treatment. Interestingly, despite only serine was encoded in position 282 during follow-up, two distinct genetic pathways for reversion were detectable. In 31% of the quasispecies the original codon for serine was present whereas in 68% of the quasispecies an alternative codon was selected. This alternative codon usage was unique for all GT3a isolates from the HCV database and remained detectable as a genetic footprint for prior resistance selection at the RNA level for at least 6 months. Comparative analyses of viral sequences at the codon level before and after DAA treatment may therefore help to elucidate the patient's history of resistance selection, which is particularly valuable for highly unfit RAS that are detectable only for a short period of time. If such codon changes increase the risk of re-selection of resistance upon a second exposure to SOF remains to be addressed.

5.10 Die Wirkung der DAA-basierten HCV-Therapien wird in vitro durch mTOR-Inhibitoren modifiziert

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Hintergrund: Die neuen direkt antiviral wirkenden Therapeutika (DAAs) ermöglichen eine Verlaufsverbesserung der bisher schlechten Langzeitprognose und hohen Hepatitis C Virus (HCV)-Reinfektionsraten nach Lebertransplantation (LT). In diesem Zusammenhang wurde der Einfluss der immunsuppressiven Therapie (IS) auf die Wirkung der DAAs nicht untersucht. Mit unseren Untersuchungen wollen wir den Einfluss der verschiedenen IS (Calcineurin (CNI)- und mTor-Inhibitoren) auf die Wirkung der verschiedenen DAAs in vitro aufklären. **Methoden:** Verschiedene HCV-Replikon-Konstrukte (GT1b, GT2a, GT3a) im Zellkulturmodell repräsentierend die unterschiedlichen HCV-Genotypen (GT) in vitro. Es erfolgte eine Behandlung der Zellen mit Kombinationen aus den IS (Everolimus (EVR), Sirolimus (SRL), Cyclosporin A (CsA) und Tacrolimus (TAC)) und den verschiedenen DAAs (Simeprevir (SIM), Sofosbuvir (SOF), Daclatasvir (DCV), Ledipasvir (LDV)). Die HCV-Replikationsaktivität wurde mittels Luciferase Assay oder qrt-PCR bestimmt. Mögliche zellproliferationsbedingte Effekt wurden mittels MTT-Assay untersucht. **Ergebnisse:** Die Behandlung der Replikonzellen mit den mTor-inhibitoren EVR und SRL führt zu einer um 20% verstärkten antiviralen Wirkung von SOF ($p \leq 0{,}01$). Auch die Kombination der mTor-Inhibitoren mit SIM, DVC oder LDV resultiert in einer um bis zu 30% gesteigerten antiviralen Aktivität ($p \leq 0{,}01$). Die CNIs CsA und TAC haben hingegen keinen Einfluss auf die antivirale Wirkung der verschiedenen DAAs. **Schlussfolgerungen:** Die antivirale Wirkung der DAAs wird durch Immunsuppressiva in vitro beeinflusst. mTor-Inhibitoren verstärken dabei die antivirale Wirkung in Abhängigkeit von der antiviralen Substanz und dem Genotypen deutlich, wohingegen die CNIs keinen Effekt auf die antivirale Wirkung der DAAs zeigten.

5.11 Different antiviral effects of IFN-αlpha subtypes in a mouse model of HBV infection

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Interferon alpha (IFNα) is commonly used for the treatment of chronic hepatitis B (CHB) patients. There are 13 different IFNα subtypes in humans, but only the subtype IFNα2 is used for clinical treatment. The antiviral activities of all other IFNα subtypes against HBV have not been studied. To obtain basic knowledge about the direct antiviral as well as the immunomodulatory effects of IFNα subtypes, we used the HBV hydrodynamic injection (HI) mouse model. Application of most IFNα subtype proteins inhibited HBV replication in vivo, with IFNα4 and IFNα5 being the most effective subtypes. Decreased viral loads after therapeutic application of IFNα4 and IFNα5 correlated with expanded effector cell populations of NK cells and T cells in both liver and spleen. Hydrody-

namic injection of plasmids encoding for the effective IFNα subtypes (pIFNα) was even more potent against HBV than injecting IFNα proteins. The combination of pIFNα4 and pIFNα5 showed a synergistic antiviral effect on HBV replication, with a strong increase in NK cell and T cell activity. The results demonstrate distinct anti-HBV effects of different IFNα subtypes against HBV in the mouse model, which may be relevant for new therapeutic approaches.

5.12 Diversity of clinical presentation and virological characteristics of hepatitis delta: The hepatitis Delta International network (HDIN)

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Background and Aims: The hepatitis delta virus (HDV) causes the most severe form of chronic viral hepatitis associated with fibrosis progression and an increased risk for developing liver-related clinical complications. The clinical and virological presentation of hepatitis delta patients varies largely between different regions and countries. The hepatitis Delta International network (HDIN) was established in 2011 to realize a previously unmet research need as there was no international platform to systematically collect data from hepatitis delta patients. The primary aim of the HDIN registry is to define the course of hepatitis delta and response to antiviral therapy in the context of different HDV and HBV genotypes and diverse host genetic and environmental backgrounds. **Methods:** The registry was designed by the HepNet Study-House of the German Liver Foundation in close collaboration with clinicians and researchers in Europe, Asia, North and South America. The HDIN is funded by the German Center for Infection research DZIF. A structured eCRF optimized for hepatitis delta was implemented and 14 centers worldwide are participating. A central data monitoring process has been established. We here report data of more than 1300 patients included until Nov 1st 2015. **Results:** Patients were mainly male (64%) with a median age of 37 years. 81% of patients were HDV RNA positive and 76% were HBeAg-negative. Hematological and biochemical parameters widely varied. The severity of hepatitis delta was confirmed as 447 patients (33%) had liver cirrhosis and about one quarter of patients presented low platelet counts, elevated INR and/or low albumin. Furthermore 18% of patients were icteric. Previous decompensation defined as ascites, oesophageal bleeding and encephalopathy occurred in 219 patients (16%). Hepatocellular carcinoma was detected in only 37 patients (2.7%). Liver transplantation was necessary in 70 patients (5%). Patients were divided according to the country of birth into Eastern Mediterranean EM (13%), Eastern Europe and Central Asia EE/CA (42%), Central and Southern Europe CE (5%), South Asian SAS (mainly Pakistan; 21%) and South America SA (19%). Patients from EE were the youngest and had the highest biochemical disease activity. While patients from SA showed the lowest platelet counts and the highest INR. In SA over half of the patients developed a clinical event, whereas in other regions endpoints were developed in about 15% of patients only. Previous antiviral therapy was reported for only 27% of patients with large differences between the regions with treatment rates between 62% in SA and 33 – 35% in EM and EE and only 11% in SAS. The majority of patients were treated with IFN (63%). **Conclusions:** The HDIN registry highlights the diversity of patient characteristics in different regions world-wide and confirms the severity of chronic hepatitis delta. There is an urgent need for new treatment possibilities but also probably requiring different management strategies.

5.13 Effect of Hepatic Cirrhosis on the HCV Replication Cycle

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Introduction: Worldwide an estimated 160 million people suffer from chronic hepatitis C (CHC). In about 20% of cases CHC progresses to severe liver disease, i.e. cirrhosis or hepatocellular carcinoma (HCC) within 20 years. Cirrhosis leads to alterations in the hepatic microenvironment as well as a number of metabolic and hormonal changes. The effects of these alterations on the HCV replication cycle have not yet been investigated. **Aim:** The aim of the study is to determine whether the HCV replication cycle is affected by the metabolic changes due to the progression of cirrhosis. **Methods:** The study included 48 serum samples from patients diagnosed with liver cirrhosis of the stages Child-Pugh A, B and C as well as healthy controls. All participants were HCV negative. We tested whether the addition of healthy and cirrhotic sera differentially affects the different steps of the HCV replication cycle in vitro. All experiments were performed in the presence of 10% human serum. For the analyses retroviral HCV pseudoparticles, HCV subgenomic replicons and fully infectious virions of the genotypes 1 and 2 carrying a luciferase reporter gene were used. **Results:** The sera of Child B and C individuals showed a significant inhibitory effect on HCV infectivity using full length fully infectious HCV. Effects on viability and/or cell proliferation of the cells used were not detected so that a specific modulation of the HCV replication cycle was considered likely. Upon dissection of individual replication cycle step, we found that both cell entry and replication of HCV in vitro was impaired in the presence of sera from individuals with more advanced liver disease compared to sera of healthy controls and patients with Child A cirrhosis. However, cell entry effects appeared to be partially dependent on the cell line used. We hypothesized that lower levels of branched chain amino acids (BCAAs), activators of mTOR, in sera of patients with liver cirrhosis may be responsible for the observed effects. While we could confirm declining BCAA serum levels in advanced liver disease BCAA supplementation was unable to revert the inhibitory effect of Child C sera on HCV infectivity so that additional mechanisms appear to be involved. **Conclusion:** Our results suggest that serum of patients with advanced liver disease includes anti-viral factors or lacks pro-viral factors resulting in impaired HCV replication. Reduced replication in end-stage liver disease may impair the ability of HCV to adapt to selective pressure exerted by anti-viral therapy and thus have an unexpected favourable effect on treatment outcome in this very sick subgroup CHC patients.

5.14 Extracellular maturation of secreted hepatitis C virus particles by incorporation of Apoliporotein E enhances infectivity

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Aim of the study: Hepatitis C virus (HCV) efficiently evades humoral immune responses to establish a chronic infection. Virus particles circulate in complex with host-derived lipoproteins, thus facilitating escape from antibodies. The exchangeable apolipoprotein E (ApoE) is essential for intracellular HCV assembly as well as for HCV cell entry. To gain insights into key principles of HCV assembly and immune evasion, we explored if ApoE released from non-infected human liver cells interacts with secreted HCV particles and modulates their properties. **Materials & methods:** ApoE was expressed in human hepatoma cells and cell-free culture fluid enriched with ApoE was incubated with secreted HCV particles at ApoE doses comparable to typical levels in human sera. A direct interaction between cell culture derived and patient-derived HCV and exogenously added ApoE was quantified using an HA-tagged ApoE variant and immunoprecipitation. Infectivity of ApoE-conditioned HCV particles was determined using luciferase reporter virus and limiting dilution infection assays. **Results:** Extracellular ApoE increased infectivity of secreted HCV particles from all genotypes as well as of HCV particles produced in primary human hepatocytes. Incubation of HCV particles with HA-tagged ApoE at physiological doses resulted in co-precitation of HCV core protein with HA-ApoE indicating that ApoE is loaded onto secreted HCV particles after release from infected cells. Infection of SR-BI knockout cells and heparinase treated cells as well as competition assays with SR-BI-targeting molecules and heparin indicated that ApoE enriched particles have increased entry efficiency due to improved virus attachment to cell surface proteoglycans and not -as shown for HDL-mediated enhancement- due to an interplay with SR-BI. Finally, extracellular loading of ApoE onto HCV particles decreased neutralization efficiency of E2-protein targeting monoclonal antibodies. **Conclusions:** Our data provide evidence that secreted ApoE is incorporated into released HCV particles. Thus, secreted particles undergo maturation and remodelling to enhance infectivity and thereby facilitate evasion from antibodies.

5.15 Follicular T helper cells in patients with cholestatic liver disease – a comparative study

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Background and aims: Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are the most common cholestatic liver diseases. Autoimmunity is thought to be involved in the pathogenesis of both diseases, however, autoantibodies are more closely associated with PBC than PSC. The aim of our study was to analyze and to compare the T cell phenotypes in PBC and PSC patients, focusing on the characteristics of follicular T helper cells (Tfh cells, $PD1^+CXCR5^+$ cells of $CD4^+$T-lymphocytes). Tfh cells enable B cell maturation and antibody formation and their imbalance has been shown to cause the emergence of autoantibodies in various autoimmune diseases. **Methods:** In this cross sectional study, 18 patients with PBC and 20 patients with PSC who were treated at our Liver Unit between 2014 and 2015 were included in the study and compared to a group of 23 healthy donors. Flow cytometry analyses were performed on isolated Peripheral Blood Mononuclear Cells stained with fluorescence-marked antibodies. **Results:** Patients with PBC displayed higher numbers of T-lymphocytes ($CD3^+CD19^-$ cells, $p = 0.004$) compared to the healthy donor cohort, whereby both the $CD4^+$- as well as the $CD8^+$- subset were increased ($p = 0.028$, $p = 0.048$). Moreover, Tfh-cells were present at higher frequencies in patients with PBC ($p = 0.05$), with a significantly increased level of activation as characterized by expression of $CXCR3^+$ ($p < 0.001$) or $Ox40^+$ ($p < 0.001$). Regarding the frequency of naive $CD4^+$ lymphocytes ($CCR7^+CD45RO^-$ cells) PBC patients expressed lower frequencies. Central and effector memory cells were not affected by any differences. In addition, frequencies of gut homing lymphocytes expressing CCR9 were similar in all groups. **Conclusion:** Total T cells numbers and follicular T helper cells are increased in patients with PBC and Tfh cells display a more activated phenotype compared to those of healthy donors. In contrast, patients with PSC showed none of those features. These results suggest a role for Tfh cells in the pathogenesis of PBC but not PSC, and offer an explanation for the different roles of autoantibodies in cholestatic liver diseases.

5.16 HDV-GT3 shows similar induction of innate immunity compared to HDV-GT1 in humanized mice despite its high infection efficiency and intrahepatic activity

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Hepatitis D virus genotype 1 (HDV-GT1) is the most common genotype worldwide, while HDV-GT3 is mainly restricted to South America but is associated with a more severe outcome of the disease. **Aim:** To investigate virological differences among these two genotypes and their capacity to enhance innate responses in human hepatocytes using humanized mice. **Methods:** Human liver chimeric uPA/SCID/beige (USB) mice were co-infected with cell culture-derived HDV-GT1 or HDV-GT3 and HBV (GT-D). 9 weeks post inoculation (p.i.), HBV and HDV viremia, intrahepatic HDV RNA, pgRNA and RNA levels of interferon stimulated genes (ISGs) and cytokines were determined by qRT-PCR. Antigenomic HDV RNAs were detected by a specific RT-PCR-based assay and in situ hybridisation. HDAg was assessed by immunofluorescence. **Results:** 9 weeks p.i., mice co-infected with HBV and HDV-GT3 showed a 3.6-fold higher HDV viremia (median 2.4 × 10E8 copies/ml) and 9.3-fold higher levels of intrahepatic HDV RNA than HDV-GT1 co-infected mice. Intrahepatic analyses also revealed that the amount of antigenomic HDV RNA was 3.2-fold higher in the liver of HDV-GT3 compared to HDV-GT1 infected mice, suggesting increased virion productivity of HDV-GT3 RNAs. Also a higher number of human hepatocytes appeared HDAg-positive in mice infected with HDV-GT3. Of note, both HBV viremia and amounts of pgRNA appeared lower in mice co-infected with HDV-GT3 (median 6.1 × 10E8 copies HBV DNA/ml and median 1.9 pgRNA relative to hGAPDH) compared to those co-infected with HDV-GT1 (median 2.6 × 10E9 copies HBV DNA/ml and median 4.9 pg RNA relative to hGAPDH), indicating that HDV-GT3 can suppress HBV replication to some extent. Remarkably, both HDV genotypes induced comparable enhancement of human ISGs such as MxA (HDV-GT1: 9.0-fold; HDV-GT3: 7.9-fold induction compared to uninfected mice) and ISG15 (HDV-GT1: 13.7-fold; HDV-GT3: 12.8-fold induction) and of the cytokine CXCL10 (HDV-GT1: 5.9-fold; HDV-GT3: 3.8-fold induction). **Conclusions:** Despite its higher infection efficiency, activity and HBV suppression capabilities, HDV-GT3 showed no augmented activation of hepatocyte innate responses compared to HDV-GT1 in humanized mice. These results suggest that direct virological effects may affect HBV productivity, whereas immune cell activity shall be responsible for the more severe course of disease in HDV-GT3 infected patients.

5.17 Hepatitis B virus activates toll-like receptor 2 signaling in primary human hepatocytes

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Background: Chronic infection with the hepatitis B virus (HBV) is a major cause of liver-related morbidity and mortality world-wide. Little is known about the initial cellular response in hepatocytes upon HBV infection. Here, we analyzed the responses of primary human hepatocytes (PHH) infected with HBV in vitro. **Methods:** PHH were isolated after perfusion and digestion of liver tissue obtained after tumor resections or transplantations. Cells were infected with cell culture-derived HBV particles and cultured for ten days. Infection was monitored by the release of HBsAg and HBeAg, HBV DNA, and immunohistochemistry

staining. Cellular responses in PHH were analyzed by microarray and quantitative RT-PCR. TLR ligands and neutralizing antibodies were used to characterize the initial immune response of PHH **Results:** PHH could be efficiently infected with cell culture-derived HBV particles. The secretion of viral antigens and expression of viral RNAs in PHH could be inhibited by UV irradiation of viral particles. The HBV exposure dose-dependently induced a gene expression profile in PHH that is comparable to a TLR2-mediated response after Pam3Cys stimulation, leading to the induction of inflammatory and chemoattractant cytokines. The induction of interferons or interferon-stimulated genes in PHH was not detected neither initially nor at later time points after HBV infection. Furthermore, HBV-induced gene expression could be neutralized by TLR2-specific antibodies. Interestingly, PHH isolated from HBV-infected patients revealed a higher responsiveness to TLR2 stimulation compared to uninfected resection or transplantation controls, indicated by elevated induction of cytokine gene expression. **Conclusions:** The present data demonstrate that TLR2 may be involved in recognition of HBV during the infection process and activate cellular responses in PHH. Consistently, recent data suggest that TLR2 might be involved in antiviral responses during hepadnaviral infection and play an important role in the pathogenesis of chronic HBV infection.

5.18 HLA-Bw4 80(T) and high HLA-Bw4 copy numbers in combination with KIR3DL1 are associated with superior immune control of HCV infection in people who inject drugs

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Objective: NK cell function is regulated by inhibitory and activating receptors including killer-cell immunoglobulin-like receptors (KIRs). Here, we analyzed the impact of different KIR/KIR-ligand genotypes on the outcome of HCV infection in people who inject drugs (PWID). **Design:** The KIR/KIR-ligand genotype was determined in 266 therapy-naïve PWID including 215 anti-HCV positive PWID (151 HCV-RNA positive and 64 HCV-RNA negative) and 51 anti-HCV seronegative PWID with high risk behavior. NK cells of 90 PWID (30 of each group) and 120 healthy donors were functionally characterized by flow cytometry. **Results:** Multivariate logistic regression analysis revealed that KIR3DL1/HLA-Bw4 80 (T) was associated with an HCV-RNA negative status in anti-HCV seropositive PWID. Moreover, the frequency of individuals with multiple HLA-Bw4 alleles was significantly higher among anti-HCV seropositive PWID with undetectable HCV-RNA (29.7%; $p = 0.0229$) and even more pronounced in anti-HCV seronegative PWID (39.2%; $p = 0.0006$) compared with HCV-RNA positive individuals (15.2%). In line with the genetic association KIR3DL1+ NK cells from HLA-Bw4 80(T)-positive PWID showed superior functionality compared to HLA-Bw4 80(I)-positive PWID. This differential impact of HLA-Bw4 80(T) on NK cell function was not observed in healthy donors, however, here, the HLA-Bw4 copy number strongly correlated with the functionality of KIR3DL1+ NK cells. **Conclusions:** HLA-Bw4 – 80(T) and multiple HLA-Bw4 copies in combination with KIR3DL1 are associated with protection against chronic hepatitis C in PWID by distinct mechanisms. Better licensing of KIR3DL1+ NK cells in the presence of multiple HLA-Bw4 copies is beneficial prior to seroconversion whereas HLA-Bw4 80(T) may be beneficial during acute hepatitis C.

5.19 Identification of host cell requirements and antivrial targets for hepatitis D virus infection

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Hepatitis delta is the most severe form of viral hepatitis. It is caused by the hepatitis delta virus (HDV), a co-infecting agent of hepatitis B virus (HBV). Though, successful vaccines against HBV exist, about 350 mio people are globally infected with HBV and of these 15 – 20% have chronic hepatitis D. Cure rates are below 25% and the only treatment option so far is pegylated interferon alpha. To study HDV infection and replication in vitro, we developed a semi-automated setup. We used the HuH7/hNTCP cell line, stably overexpressing the HBV/HDV entry receptor human NTCP (sodium-taurocholate co-transporting polypeptide). Experiments were performed in the 96-well plate format. To detect infection results, immunofluorescence staining using a novel monoclonal anti-HDAg antibody was applied. The cells were read using an automated microscope. The cell profiler software was used to quantify all cells and infection events. With this method we screened a library of 160 human kinase inhibitors. Our experiments showed that kinase groups AGC, CMGC, TK, and TKL seemed to play a role in the HDV infection and replication process. Specifically, kinase inhibitors HA 1077 (Dihydrochloride Fasudil), Cdk/Crk inhibitor, Cdk2 inhibitor III, PD 174265, Syk inhibitor III, IRAK-1/4 inhibitor, GSK-3β inhibitor I, and Kenpaullone showed a decreasing effect on HDV infection and replication. HA1077, for example, is approved for treatment of cerebral vasospasm in Japan and China. It inhibits dose-dependently PKA, PKG, MLCK, but its main target is ROCK. The Rho-activated kinase ROCK belongs to the AGC group and mainly acts on the actin cytoskeleton and is thus a known target for viral modifications. Defining selective indices for the eight drugs showed that the values were rather low, the highest selective index was present for Kenpaullone, SI 3.44. Thus, we do not present a likely new therapeutic lead, but the introduced assay offers a useful medium throughput platform for screening inhibitors of HDV entry and replication.

5.20 IL-18, CXCL-8 and CXCL-10 plasma levels decrease in patients with chronic Hepatitis C virus infection undergoing DAA therapy

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The recently approved treatment of chronic Hepatitis C virus infection (cHCV) with direct acting antivirals (DAA) leads to sustained virological response rates (SVR) of more than 90%. The elimination of the virus leads to a subsequent decline of chronic inflammation. Important inflammatory mediators are cytokines that show differential profiles in cHCV patients compared to healthy controls. Until now, the effect of successful virus elimination by DAA therapy on the cytokine milieu has not been comprehensively investigated. In this study, we therefore performed longitudinal analyses of cytokine profiles in the plasma of DAA-treated patients with cHCV. In detail, 26 cytokines in the plasma of 37 patients with cHCV were quantified by enzyme-linked immunosorbent assay (ELISA) and cytometric bead arrays. All patients received DAA therapy (n = 22 Harvoni, n = 7 Harvoni/Ribavirin, n = 3 Viekirax/Exviera, n = 3 Viekirax/Exviera/Ribavirin, n = 2 Daklinza/Sovaldi) and achieved an SVR 12. Liver cirrhosis was present in 12 of the examined patients. Cytokine profiles were analyzed at therapy baseline (W0), during therapy (W4), at the end of therapy (EOT) and in a follow-up examination (12 – 28 weeks after end of therapy; FU). 31 healthy donors served as control group. While most of the analyzed cytokines including the anti-inflammatory cytokines IL-10 and TGF-β remained stable, plasma levels of the pro-inflammatory cytokine IL-18 declined during treatment. This decline in IL-18 indicates extensive changes of consequent immune responses since IL-18 is an important functional regulator of innate and adaptive immunity by inducing IFN-γ production in different cell types. Furthermore, the chemokines CXCL-8 and CXCL-10 decreased until they reached levels similar to healthy donors clearly suggesting a re-orchestration of innate and adaptive immune cells with potential consequences for inflammatory processes. This assumption was further supported by the

observed correlation of CXCL-8 with transaminases during DAA treatment. In sum, we observed dynamic changes in plasma cytokine profiles of DAA-treated cHCV patients that were also linked to inflammatory parameters. These results give novel insights into the complex changes in systemic viral induced immune responses during and after chronic infection.

5.21 Immunosuppressive effects of bile and bile acids on natural killer cell activity

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Objective: Patients with severe liver diseases have been demonstrated to display low natural killer (NK) cell activity. In this context, it is important to note that bile acids have been shown to be involved in cellular immunological processes and to suppress cell-mediated innate and adaptive immunity. Here, we investigated the effects of human bile and bile acids on phenotype and function of human conventional NK cells (cNK). **Methods:** Freshly isolated cNK cells form healthy donors [n = 10] were incubated in bile (diluted 1:1000/500/250/100) or the bile acids ursodeoxycholic acid (UDCA) and chenodeoxycholic acid (CDCA) (50/100/200 µM/mL) for 15 hours. Then, cNK cell phenotype as well degranulation (CD 107a) and IFN-γ was studied following incubation with either K562 or the human duodenal adenocarcinoma cell line HuTu80 using flow cytometry. **Results:** We found incubation of healthy cNK cells in human bile or CDCA to result in a significantly decreased expression of the activating NK cell receptors NKp46 and NKG2D. Moreover, cNK cell activation, assessed by measuring CD 69 expression, was significantly reduced in bile and bile acid treated cNK cells. More importantly, we found both cytotoxic activity against HuTu80 cells as well as cNK cell IFN-γ production to be significantly decreased following incubation in the presence of bile/bile acids. **Conclusion:** Our data suggest the human bile and bile acids can impair cNK cell functions which might contribute to the immunpathogenesis of liver disease.

5.22 Lack of NKp80 expression define a liver-resident CD 49a(+) NK cell subset with ILC 1-like features

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Background: The human liver contains a unique CD 49a+ NK cell subset that resembles murine liver-resident NK cells. At the moment it is not fully understood if these cells have conventional NK cell or ILC-(innate lymphoid cells) type 1-like features. For this reason we have analyzed liver CD 49a(+) NK cells in more detail and compare them with similar CD 49a(+) cells from the gastrointestinal (GI) tissues. **Material and Methods:** Human tissue-infiltrating lymphocytes were isolated from 15 liver-perfusates, 12 -resections, 18 -explants, 29 -biopisies and 33 biopsies from normal gastrointestinal tissues. Lymphocytes were phenotypically characterized by multicolor flowcytometry and tested for cytokine production following PMA/ionomycin stimulation. **Results:** Analyzing liver infiltrating CD 49a(+) CD 94(+) CD 56(+)CD 3(-)NK cells, we found 2 distinct subsets defined by expression of the NK cell activating receptor NKp80. Unlike CD 49a(+)Nkp80(+) cells, the CD 49a(+)NKp80(-) subset is negative for the transcription factor EOMES and express the cytolytic effector molecule perforin at low levels. Markers for tissue resident lymphocytes like CD 69 [NKp80(-): 38% vs. NKp80(+): 35%] and CD 103 [NKp80(-): 39% vs. NKp80(+): 31%] are expressed on both CD 49a(+) subsets. The capacity to produce IFNγ do not vary between these cells. Surprisingly, frequency of CD 49a(+)Nkp80(-) cells is very low in liver tissue (0.3% lymphocytes) and is not significantly different to CD 49a(+) Nkp80(+)(0.63%) or to liver ILC 1/ILC 2/ILC 3 (0.64%). CD 49a(+)NKp80(-) CD 94(+) NK cells could also be detected in gastrointestinal tract: stomach (0.8% of lymphocytes) and duodenum (0.5%) have significantly higher frequencies of this subset, whereby frequencies in esophagus (0.2%), ileum (0.3%) and colon (0.3%) are comparable to liver tissues. CD 49a(+)NKp80(-) CD 94(+) NK cells from GI tract are abundant for expression of CD 103, CD 69 and NKp44. Recently, these cells were described as CD 103(+) intraepithelial ILC 1. **Conclusion:** Liver CD 49a(+) NKp80(-) NK cells share some features of non-conventional NK cells and are maybe a counterpart of mucosal CD 103(+) intraepithelial ILC 1.

5.23 Macrophage-derived extracellular vesicles mediate a long-lasting innate immune response against hepatitis C virus

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Background: Interferons play a pivotal role in the first line defense against viral infections. Macrophages are both important producers and responders to type I and II interferons and participate in antiviral immune responses. Yet, the precise role of macrophages in the immune response against hepatitis C virus (HCV) has not been entirely elucidated. In the present study, we therefore aim to further characterize mechanisms of macrophage-mediated antiviral immune responses against hepatitis C virus (HCV). **Methods:** THP-1 cells and monocyte-derived macrophages from human buffy coats were stimulated with type I and II interferons. Whole supernatants and purified extracellular vesicles secreted from interferon-pulsed macrophages were transferred to Huh-7.5 cells harboring subgenomic HCV replicons. HCV replication was quantified by quantitative PCR. Macrophage-derived supernatants and extracellular vesicles were analyzed by cytokine arrays, RNA-sequencing, flow cytometry and electron microscopy. **Results:** Macrophages secrete a variety of cytokines shortly after stimulation with type I and II interferons, which orchestrate a fast but short-lasting antiviral state against HCV. In addition, interferon-pulsed macrophages secrete extracellular vesicles which induce a late, but long-lasting (5 – 7 days) inhibitory effect on HCV replication. Though extracellular vesicles of both THP-1 cells and primary human macrophages exhibit antiviral qualities, they are different with respect to vesicle size and content of extracellular vesicle markers. Preliminary results indicate that long-coding RNAs may mediate the long-lasting antiviral effects of macrophage-derived extracellular vesicles. **Conclusion:** Macrophage-derived extracellular vesicles mediate long-lasting inhibitory effects on HCV replication in vitro, which may bridge the time until efficient adaptive immune responses are established.

5.24 NK cell responses during direct-acting antiviral treatment of chronic hepatitis C virus infection

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Chronic viral infections, such as chronic hepatitis C virus (HCV) infection, are known to cause immune cell exhaustion, including alterations in NK cell phenotype and function. Novel interferon-free treatment strategies against HCV, based on direct-acting antivirals (DAAs), offer the opportunity to study whether rapid elimination of a chronic virus leads to restoration of the phenotype and functional capacity of NK cells. Here, we followed 26 chronic HCV patients before, during, and after DAA treatment. Out of these patients, two-third cleared the virus whereas a third experienced a viral relapse after treatment cessation. We show that viral loads and levels of liver enzymes rapidly declined upon DAA treatment initiation and that this was accompanied with fluctuating dynamics in absolute lymphocyte counts as well as NK cell counts. We further observed changes in NK cell surface marker expression comparing chronic HCV patients at baseline with healthy controls, including lower NK cell diversity. Further phenotypic alternations occurred during the course of DAA treatment. However, the NK cell diversity remained low even after the virus had been cleared. Intriguingly, NK cells from patients that went on to clear HCV after DAA treatment exhibited a higher level of activation and were more potent producers of cytokines as compared to NK cells from patients experiencing a viral relapse. Taken together, the NK cell phenotype and function improves during successful DAA treatment.

However, lower NK cell diversity appears to be a persistent feature after recovery from chronic HCV infection.

5.25 SEC14L2 is not a reliable predictor of HCV replication fitness

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The replication of natural patient derived HCV strains in cell culture had been an elusive target until recently Saeed et al reported on a model where the expression of the protein SEC14L2 mediated protection against lipid peroxidation enabling the replication of patient derived virus in cell culture. We employed this model to investigate the role of SEC14L2 variability in the HCV life cycle in in vitro conditions. For this purpose we generated 13 different Huh-7.5 human hepatoma cell lines, each harbouring the one of the 13 most prevalent SEC14L2 SNPs described in the literature. We infected these cell lines with patient derived serum and quantified the HCV load after infection using qRT-PCR. We show in these assays that although relevant for the HCV life cycle SEC14L2 is not a reliable predictor of HCV replication fitness. Furthermore we show evidence for a different susceptibility to lipid peroxidation from different genotypes which argues in favour of different mechanisms from HCV to cope with host cell ROS, which is compatible with previous reports where different HCV genotypes would differentially interact with the host lipid metabolism.

5.26 Single Nucleotide Polymorphisms (SNPs) im Scarb1 Gen und deren Bedeutung für eine Hepatitis C Virus Infektion

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Hintergund: Scavenger receptor class B type I (SR-BI) ist der physiologische Rezeptor für das High Density Lipoprotein (HDL) und ein essentieller Hepatitis C Virus (HCV) Eintrittsfaktor. SR-BI wird vom Scarb1 Gen kodiert und eine Vielzahl sogenannter single nucleotide polymorphisms (SNPs) die mit einem klinischen Phänotyp wie der Beeinflussung der Lipidspiegel im Serum assoziiert sind, wurden in der Literatur beschrieben. Bisher ist deren Bedeutung für eine HCV Infektion jedoch ungeklärt. **Methoden:** Um die Bedeutung für die HCV Infektion näher zu untersuchen wurden kodierende und nicht-kodierende SCARB1 Varianten ausgewählt. Kodierende nicht-synonyme SNPs wurden in einer SR-BI negative Hepatomazelllinien (7.5/TS4-B2), die mithilfe von CRISPR/Cas generiert wurde, auf deren Fähigkeit als HCV Rezeptor zu fungieren in vitro getestet. Zur Untersuchung der synonymen Varianten wurden vier häufig auftretende SNPs, die mit einem klinischen Phänotyp assoziiert sind, in genetischen Assoziationsstudien in einer gut charakterisierten Studienkohorte chronisch HCV infizierter Patienten untersucht. Parallel dazu wurde die SR-BI Expression in Lebergewebe via Immunoblot analysiert und eine Korrelation zu den jeweiligen SCARB1 Genotypen untersucht. **Ergebnisse:** Die Expression der nicht-synonymen Varianten in 7.5/TS4-B2 Zellen zeigte eine verminderte Infektiosität für die SNPs S112F und T175A im Vergleich zu Wildtyp SR-BI. Die verbleibenden kodierenden Varianten (G2S, V135I, P297S) wiesen eine vergleichbare Infektiosität wie Wildtyp SR-BI auf. Weiterhin war die Bindung von löslichen E2 und authentischen HCV für die beiden Varianten S112F und T175A reduziert. Eine Reduktion der Zelloberflächen Expression für SR-BI Varianten konnte nicht nachgewiesen werden. Ein off-target Effekt des CRISPR/Cas Konstrukts auf den Low Density Lipoprotein Rezeptor war nicht zu beobachten. Innerhalb der nicht-kodierenden Varianten fanden wir, dass homozygote Träger des G Allels für SNP rs3782287 mit einer signifikant niedrigeren Viruslast assoziierte waren. Außerdem zeigte sich ein Trend für eine verminderte SR-BI Expression in Lebergewebe vom Patienten mit einem GG Genotyp. Haplotyp Analysen bestätigten dieses Ergebnis und identifizierten einen Haplotyp, der eine Vorhersage zur Viruslast ermöglichen. **Schlussfolgerung:** Die Untersuchungen belegen, dass sowohl kodierende wie auch nicht-kodierenden SNPs im Scarb1 Gen eine direkten Einfluss auf den HCV Replikationszyklus haben können: häufig auftretende synonyme SNPs auf die virale Resistenz während die seltenen kodierenden Varianten mit einer zellulären Resistenz gegenüber dem HCV Zelleintritt assoziiert sein können. Die Ergebnisse zeigen die Relevanz von SR-BI als HCV Rezeptor und erlauben ein besseres Verständnis hinsichtlich des individuellen Verlaufs einer HCV Infektion.

5.27 Single-Nucleotide Polymorphism in HLA DPA1 Gene Associates with Occult Hepatitis B Infection (OBI) in Indonesian Blood Donors

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Background: A recent genome-wide study showed that several Single-Nucleotide Polymorphisms (SNPs) in Human Leukocyte Antigen (HLA)-DPA1/DPB1 gene were associated with Hepatitis B Virus (HBV) infection, including susceptibility, persistent infection, or disease progression. We focused on the SNPs in HLA gene in relation to Occult Hepatitis B Infection (OBI) among Indonesian population. **Method:** 456 healthy participants (380 men and 76 women; age 29.95 ± 9.78 years) with HBV surface antigen (HBsAg) negative were collected in Yogyakarta, Indonesia. The anti-HBs and anti-HBc antibodies were assayed with chemiluminescence immunoassays (CLIA). OBI was determined by the detection of HBV-DNA using nested PCR in at least two regions out of four ORF among samples with anti-HBs and/or anti-HBc positive (seropositive). SNPs in HLA-DPA1/DPB1 were genotyped using real-time TaqMan® genotyping assays. **Result:** 122 cases (26.75%) were detected by anti-HBs and/or anti-HBc antibody. Among them, 17 cases were shown as OBI and compared with cases without OBI. As for the seropositive cases, the prevalence of minor allele of rs3077 in HLA-DPA1 gene among OBI was significantly higher than that among cases without OBI (59% and 33%, respectively). In addition, this minor allele was associated with increasing risk of OBI detection (OR 3.87, 95% CI 1.58 – 9.49, P = 0.0015, additive genetic model). There is no significant difference of age and gender between cases with OBI and without OBI among seropositive samples (P = 0.136, P = 1.000, respectively). **Conclusion:** Our result suggests that variation in HLA-DPA1 gene was associated with increased probability of OBI detection in Indonesian Blood Donors. Key word: HBV, OBI, HLA-DP variants, Indonesian Blood Donors.

5.28 Subset diversification of virus-specific CD8+ T-cells in chronic HCV monoinfection and HCV/HIV coinfection

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Chronic viral infections like Hepatitis C virus (HCV) and Human immunodeficiency virus (HIV) lead to considerable morbidity and mortality worldwide. HIV/HCV coinfection is very common among certain risk groups and has significant clinical and therapeutical impacts, e.g. faster progression of HCV-related liver disease compared to HCV monoinfection. Immunologically, the persistent antigen exposure in both chronic HCV and HIV infection induces CD8+ T-cell exhaustion that is characterized by impaired functional capacity e.g. reduced proliferation, cytotoxicity and cytokine production, increased co-expression of inhibitory re-

ceptors and a distinct transcriptional profile. An increasing body of data from the mouse model of chronic Lymphocytic choriomeningitis virus (LCMV) infection has unraveled progenitor-progeny heterogeneity among exhausted virus-specific CD8+ T-cells. However, in human viral infection little is known about exhausted virus-specific CD8+ T-cell subsets. In this study, we therefore focused on subset diversification of exhausted virus-specific CD8+ T-cells in HCV monoinfection compared to HIV/HCV coinfection. For this, we analyzed HCV- specific CD8+ T-cells of chronically HCV-infected patients (n = 20) as well as HCV- and HIV-specific CD8+ T-cells of HIV/HCV coinfected patients (n = 7) and of HIV-infected patients with resolved HCV (n = 4). Following a peptide/HLA-A*02 -tetramer-based enrichment via magnetic cell separation, virus-specific CD8+ T-cells were analyzed on a single-cell level by multicolor flow cytometry for their expression of surface receptors and transcription factors. In HCV monoinfection, we found that HCV-specific CD8+ T-cells expressing the inhibitory receptor PD1 could be subdivided into cells either expressing or lacking CD127, the IL-7R α-chain that is required for T-cell homeostasis. The CD127-PD1+ subset expressed high levels of the ectonucleotidase CD39 and the transcription factor Eomes characteristic for terminal CD8+ T-cell exhaustion. In contrast, the CD127+PD1+ HCV-specific CD8+ T-cells appear to represent a less differentiated subset characterized by expression of TCF1, a transcription factor that is associated with the proliferative capacity of CD8+ T-cells. This subset diversification in less differentiated and terminal subsets could also be found among HCV- and HIV-specific T-cells targeting single epitopes in HIV/HCV coinfection. In summary, we could identify heterogeneous subsets of HCV-specific CD8+ T-cells in chronic HCV monoinfection as well as in HCV/HIV coinfection defined by CD127/PD1 co-expression. Due to distinct characteristics of these subsets, the exact composition of virus-specific CD8+ T-cell populations has implications for CD8+ T-cell immunity during chronic viral infection, specifically for immunotherapeutic approaches that should target less exhausted T cell subsets.

5.29 Sustained Vd1 and Vd2 T-cell profiles in chronic viral hepatitis

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Gamma delta (γ/δ) T cells represent a small population of innate-like lymphocytes that harbour adaptive and innate immune features. Subsets of γ/δ T cells are defined by their usage of different T-cell receptor γ and/or δ chains and exhibit characteristic tissue distributions. In peripheral blood, for example, the majority of human γ/δ T cells express the Vδ2 chain. In contrast, in tissues like the liver, human γ/δ T cells mainly carry the Vδ1 chain. γ/δ T cells sense early danger signals and can rapidly respond to several bacterial, viral and parasitic infections. Yet, only very little is known about the role, function and phenotype of the different γ/δ T cell subsets in the context of chronic viral hepatitis. It was therefore the goal of our study to generate γ/δ T-cell profiles with regard to their functionality in chronic Hepatitis B virus (HBV) and Hepatitis C virus (HCV) infection. For this, we performed comprehensive flow cytometric analyses of peripheral blood γ/δ T cells obtained from individuals chronically infected with HBV (n = 19) or HCV (n = 20) compared to 20 healthy donors. The analyses covered activation state, functionality and differentiation including transcription factor profiles of circulating Vδ1 and Vδ2 expressing γ/δ T cells. Vδ1 and Vδ2 T-cell subsets expressed high levels of the transcription factors Tbet and Eomes that are both essential for T-cell effector functions and differentiation. However, our results also revealed striking differences between these two γ/δ T-cell subsets. In particular, almost all Vδ1 T cells but only a minor fraction of Vδ2 T cells expressed Helios. It has been shown that the transcription factor Helios is up-regulated upon conventional T-cell activation and thus possibly reflects a higher activation status of Vδ1 compared to Vδ2 T cells. In line with this, a higher frequency of Vδ1 T cells expressed activation markers such as CD69 and CD38. Interestingly, only Vδ2 T cells expressed the transcription factor PLZF in high frequencies, that is associated with an innate-like effector differentiation. This PLZF expression was accompanied by expression of CD161 and IL18Rα. In addition, Vδ2 T cells exhibited robust IFNγ and TNF production upon stimulation with IL-12, IL-15 and IL-18. Of note, this combination of phenotypic and functional features is characteristic for Mucosal-associated invariant T (MAIT) cells that also belong to innate-like lymphocytes. This similarity between Vδ2 T cells and MAIT cells may represent a safeguard mechanism of immediate immune responses by functional redundancy. Importantly, the aforementioned phenotypic and functional profiles of Vδ1 and Vδ2 T-cell subsets remained stable despite chronic infections with HBV or HCV indicating intrinsic subset-defining programmes with limited plasticity.

5.30 The impact of ERAP1 polymorphisms on virus-specific CD8+ T cell responses in an HLA*B27+ patient with acute hepatitis C virus infection

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Background/aim: In hepatitis C virus (HCV) infection, HLA-B*27 is associated with spontaneous viral clearance of acute infection. However, some HLA-B*27+ patients develop chronic HCV infection due to an inefficient HCV-specific immune response. In order to determine factors influencing this course of infection we comprehensively characterized an HLA*B27+ patient with acute infection, who controlled the virus to low titers close to the limit of detection but did not completely clear the virus for > 12 months. **Methods:** We performed a comprehensive analysis of HCV-specific CD8+ T cell responses in this patient, using overlapping peptides, epitope fine trimming and HLA class I restriction experiments. In addition, we performed genetic analysis and functional testing of the endoplasmic reticulum aminopeptidase 1 (ERAP1) genotype and phenotype of the patient. **Results:** We identified a total of 6 HCV-specific CD8+ T cell epitopes targeted in this patient. These epitopes were restricted by HLA-A*01, A*26, B*08, and B*27. Strikingly, some of these epitopes overlapped with previously described epitopes. However, most of the epitopes identified in the patient were longer than usually observed CD8+ T cell epitopes, e.g. consisting of 10 – 11 amino acids instead of 9 amino acids. This phenomenon was most obvious for a previously described HLA-B*27 restricted epitope. This epitope was described as a 9-mer previously, but targeted as an 11-mer in the patient. The endoplasmic reticulum aminopeptidase 1 (ERAP1) trims peptides in order to generate optimal ligands for HLA molecules. Polymorphisms in the ERAP1 gene are strongly associated with ankylosing spondylitis in HLA*B27+ patients and affect length, quality and quantity of peptides that are loaded onto HLA molecules and presented to CD8+ T cells. We thus speculated that ERAP1 polymorphisms might influence the epitope repertoire in our patient. Indeed, sequencing of the ERAP1 allotypes in this patient revealed polymorphisms in the ERAP1 gene which have been shown to affect trimming activity. Functional testing confirmed that the ERAP1 allotype of the patient displayed reduced peptide trimming activity in comparison to the wildtype ERAP1. **Conclusion:** Here we show for the first time that ERAP1 allotypes may have a strong impact on the HCV-specific CD8+ T cell response and may thus influence outcome of infection. These results further indicate that similar mechanisms may contribute to the protective role of HLA-B*27 in viral infections such as HCV and HIV infection on the one hand, and to immunopathogenesis of HLA-B*27 associated rheumatologic disorders on the other hand.

5.31 The metabolic signature of adaptive NK cells is maintained during chronic viral hepatitis

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The human NK-cell repertoire is highly diverse. In particular, latent HCMV infection has been shown to induce the formation of adaptive CD56dim NK-cell subsets that can be separated from conventional subsets by expression of the activating receptor NKG2C. Conventional and adaptive NK-cell subsets differ in their effector and survival characteristics with adaptive NK cells being long-lived. In general, metabolic reprogramming is important to adapt to the bioenergetic demands of effector cell function and long-term survival of immune cells. This raises the question whether adaptive and conventional NK-cell subsets differ in their metabolic signatures related to their distinct characteristics. In addition, only little is known about the impact of chronic infection on the metabolic programme of human NK cells. To address these questions we analysed the metabolic profiles of circulating conventional (NKG2C-CD56dim) and adaptive (NKG2C+CD56dim) NK-cell subsets obtained from healthy donors (n = 20) and patients chronically infected with He-

patitis B virus (HBV; n = 20) or Hepatitis C virus (HCV; n = 20). For this, we have assessed glucose uptake, mitochondrial characteristics as mitochondrial mass and membrane potential and autophagic activity by multicolour flow cytometry. Our analyses revealed that conventional NKG2C-CD56dim NK cells display a significantly higher capacity for glucose uptake compared to adaptive NKG2C+CD56dim NK cells. Adaptive NKG2C +CD56dim NK cells, however, showed a relative increase in intact mitochondria reflected by higher mitochondrial membrane potential compared to conventional NKG2C-CD56dim NK cells while mitochondrial mass and autophagic activity were similar. Importantly, these metabolic signatures of conventional NKG2C-CD56dim NK cells versus adaptive NKG2C+CD56dim NK cells was maintained and not altered during chronic infection with HBV and HCV. In sum, our data demonstrate that conventional NKG2C-CD56dim NK cells use glycolysis to meet the bioenergetic demands suggesting a non-quiescent state of ongoing cell turnover with the requirement of increasing the biomass. In contrast, adaptive NKG2C+CD56dim NK cells represent metabolically quiescent subsets as reflected by a high fraction of cells with intact mitochondria, that is probably favourable for long-term survival. Of note, human conventional and adaptive NK-cell subsets seem to be metabolically reprogrammed on an intrinsic level since the different inflammatory environments in chronic HBV and HCV infection do not affect metabolic signatures.

5.32 Virus-specific antibodies allow viral replication in the marginal zone, thereby promoting CD8+ T-cell priming and viral control

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Clinically used human vaccination aims to induce specific antibodies that can guarantee long-term protection against a pathogen. The reasons that other immune components often fail to induce protective immunity are still debated. Recently we found that enforced viral replication in secondary lymphoid organs is essential for immune activation. In this study we used the lymphocytic choriomeningitis virus (LCMV) to determine whether enforced virus replication occurs in the presence of virus-specific antibodies or virus-specific CD8+ T cells. We found that after systemic recall infection with LCMV-WE the presence of virus-specific antibodies allowed intracellular replication of virus in the marginal zone of spleen. In contrast, specific antibodies limited viral replication in liver, lung, and kidney. Upon recall infection with the persistent virus strain LCMV-Docile, viral replication in spleen was essential for the priming of CD8+ T cells and for viral control. In contrast to specific antibodies, memory CD8+ T cells inhibited viral replication in marginal zone but failed to protect mice from persistent viral infection. We conclude that virus-specific antibodies limit viral infection in peripheral organs but still allow replication of LCMV in the marginal zone, a mechanism that allows immune boosting during recall infection and thereby guarantees control of persistent virus.

Namenverzeichnis