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1. Fibrogenesis and Nonparenchymal Cells

1.1 Intravital real time imaging of liver damage and regeneration by functional two-photon microscopy

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Recently, we have established an intravital two-photon based imaging technique that allows real-time observation of the living mouse liver. Videos can be taken at subcellular resolution (200 nm) but also the imaging of overviews over several lobules is possible. This technique gave us already surprising insight into liver disease and hepatotoxicity. In this presentation, videos will be shown illustrating the morphological key events how paracetamol (APAP) damages liver tissue. The earliest event, approximately 20 min after APAP injection, was loss of mitochondrial activity of pericentral hepatocytes as evidenced by the rhodamine dye, TMRE. Compromised mitochondrial activity was immediately followed by a widening of the adjacent bile canaliculi. Approximately 1 h later the widened bile canaliculi invaginated into the hepatocytes, initially forming approximately 1 – 2 µm large protrusions which later increased up to approximately 50% of the respective hepatocyte volume. About 2 h after APAP injection hepatocytes with bile canalicular protrusions lost their ability to secrete bile acids, leading to irreversible cell death as evidenced by propidium iodide uptake. Immune cell infiltration was observed as soon as mitochondrial membrane potential was lost. Cell type specific fluorescent reporter mice illustrate early (approximately 30 min after APAP injection) infiltration of neutrophils which after attaching to the sinusoids invade the TMRE negative hepatocytes. Only approximately 48 h later, Kuppfer cells enter the dead cell area. Although work for a formal proof is still in progress, it seems as if the infiltrating green fluorescent Kupffer cells interact with and finally phagocytose stellate cells in the dead cell area. This stimulated us to deplete Kupffer cells either by single or repeated doses of clodronate over 12 days. Interestingly, clodronate abolished stellate cells disappearance which normally takes place between days 3 and 6 after APAP administration, and induced fibrosis which was never observed when APAP was administered without clondronate. In conclusion, the direct observation of cellular and subcellular events in the living liver allows insights into the sequence of pathophysiological events which are difficult to obtain by conventional methods.

1.2 Neprilysin controls the switch between hepatic vasoconstriction and fibrogenesis

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Background: The Renin Angiotensin System (RAS) regulates portal hypertension and hepatic fibrogenesis. One component of the RAS is the zinc metalloproteinase Neprilysin (NEP), which cleaves Angiotensin I in Angiotensin 1 – 7, a counterplayer of AngiotensinII (AngII) and a vasodilator through stimulation of the MasR. On the other side NEP cleaves also Neuropeptide Y (NPY), which is a known cotransmitter of α1-adrenoceptor. In NEP-/- mice we investigated the development of portal hypertension, hepatic fibrosis and the hepatic expression levels of these vasoactive systems. **Methods:** In wild type (wt) and NEP-/- mice the portal pressure was invasively measured. Protein expression levels of vasoactive pathway components were analyzed in liver homogenates by western blotting in NEP-/- mice and compared to wt mice. mRNA expression levels of RAS components were investigated in livers by qRT PCR. The hydroxyproline content was analyzed in livers of NEP-/- and their control mice. Additionally immunohistological stainings for αSMA were performed in livers of NEP-/- and wt mice. **Results:** NEP deficient mice show a higher portal pressure compared to wt mice. The expression of Renin, AT1R downstream pathway (pJAK2, RhoA, ROCK, mDia) were increased in NEP-/- mice compared to wt mice. Moreover, livers of NEP-/- mice showed increased levels of NPY. Immunohistological stainings showed more αSMA in livers of NEP-/- mice than in wt livers. Interestingly, less hydroxyproline and collagen accumulation, assessed by Sirius red staining, were found in NEP-/- mice. Similarily, hepatic mRNA levels of collagen1 and TGFβ1 were lower in NEP-/- mice compared to wt mice. Also hepatic proliferation markers, as PCNA and Ki67, were significantly lower in NEP deficient mice compared to wt mice. This might be due to downregulation of Raf-Erk pathway in livers of NEP-/- mice. **Discussion:** NEP deficiency induced spontaneous portal hypertension without liver fibrosis due to contraction of hepatic vascular smooth muscle cells mediated by vasocontractive proteins (e.g. NPY, AngII). Importantly, collagen accumulation and cellular proliferation was decreased by NEP deficiency. Therefore, Neprilysin controls the switch between hepatic contraction and fibrosis.

1.3 Targeting liver fibrosis by siRNA-mediated inhibition of Cyclin E1 in mice

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Background & Aims: Initiation and progression of liver fibrosis requires proliferation and activation of resting hepatic stellate cells (HSC). Cyclin E1 is the regulatory subunit of the Cyclin-dependent Kinase 2 (Cdk2) and a key factor for initiation of cell cycle re-activation. We have recently shown that genetic inactivation of Cyclin E1 prevents activation, proliferation and survival of HSC and protects from liver fibrogenesis. The aim of the present study is to translate these findings into pre-clinical application by inhibiting Cyclin E1 using a siRNA based approach. **Methods:** The efficiency of several siRNA molecules for inhibiting Cyclin E1 were tested in murine and human HSC cell lines and primary HSC in vitro. For in vivo applications, we used stabilized anti-Cyclin E1 siRNA in combination with a novel liposome-based carrier, which was applied via mild tail vein injection in C57BL/6 wildtype mice. Functionality of anti-Cyclin E1 siRNA in vivo was tested after Carbontetrachloride (CCl4)-mediated acute liver injury and in fibrotic livers following 4 week CCl4 treatment. **Results:** Murine (GRX) and humane (LX2) HSC cell lines were transfected with anti-Cyclin E1 siRNA resulting in efficient inhibition of Cyclin E1. Reduced Cyclin E1 expression resulted in diminished proliferation and increased cell death as confirmed by FACS analysis, immunoblot and immunofluorescence staining of cell cycle markers. Importantly, comparable results were obtained using primary murine HSCs. Delivery of siRNA in mice was optimised and quantified using fluorescently labelled scrambled siRNA revealing a transduction rate of approximately 95% in HSC, 75% in hepatocytes, and 50% of CD45 positive cells after single injection. Acute CCl4 injury substantially induced endogenous Cyclin E1 expression; however, pre-treatment with anti-Cyclin E1 siRNA reverted Cyclin E1 expression to baseline levels of healthy mice. Moreover, Cyclin E1 inhibition was associated with significantly reduced serum transaminases and impaired proliferation of hepatocytes and non-parenchymal liver cells. In the chronic CCl4 model, weekly injection of anti-Cyclin E1 siRNA significantly limited fibrosis as shown by inhibition of septum formation and overall reduced liver injury as determined by ALT levels and liver histology. **Conclusion:** Cyclin E1 is a key mediator of liver fibrogenesis. Our present results support data in genetic Cyclin E1 knockout models and demonstrate for the first time that a pre-clinical targeting of Cyclin E1 using stabilized siRNA is feasible and has high antifibrotic therapeutic potential.

1.4 A computational multi-scale model for the integration of paracrine stimuli activating NF-kB signalling in hepatocytes after lipopolysaccharide (LPS) treatment

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Background: Bacterial lipopolysaccharide (LPS) efficiently stimulates the secretion of tumor necrosis factor (TNF)-α from non-parenchymal Kupffer cells (KCs), liver sinusoidal endothelial cells (LSECs), and hepatic stellate cells (HSCs). Paracrine-acting TNFα subsequently activates the canonical NF-kB pathway in hepatocytes, which is a central regulator of acute phase protein expression and the inflammatory response. However, the impact of individual liver cell types on this pro-inflammatory response has not been sufficiently analysed i.a. due to technical limitations. To establish a holistic view on this complex multi-scale process in a quantitative and time-resolved manner, systems biology serves as a valuable tool. **Methods:** The cellular response of murine hepatocytes upon different TNFα amounts (0.1 – 50 ng/ml) was measured by quantitative Western immunoblotting (8 time points, after 0 to 240 min) using antibodies detecting p65, IkBα, p38, and MSK1 as well as the respective phosphorylated isoforms. Using primary KCs, LSECs, and HSCs the secretion of TNFα was measured after treatment with LPS (0.5 – 100 ng/ml) for up to 24 hours (Luminex). To understand the inter-cellular communication process, we utilised a multi-scale ordinary differential equation (ODE)-based mathematical model (including 33 variables, 59 reactions) combining quantitative and time-resolved data. **Results:** Based on experimental results, we established a hepatocyte-specific mathematical model for TNFα-induced NF-κB signalling, which accurately described the dynamic behaviour of NF-kB pathway activation, target gene expression, protein turnover, p65/IkBα complex formation, TNFα dose-dependence, as well as activation of the p38/MSK-1 axis. Quantitative TNFα secretion profiles of KCs, LSECs, and HSCs upon LPS stimulation revealed that LSECs and KCs secreted similar high cytokine amounts (up to 0.026 µM and 0.023 µM, respectively), while HSC-derived TNFα was much lower (constitutive mean 0.004 µM). Subsequently, NF-kB model simulations using the TNFα input derived from LSECs and/or KCs were computed individually or in combination. The results clearly illustrated that low concentrations of LSEC- and KC-derived TNFα (up to 0.12 ng/ml), which were induced by low LPS amounts (from 0.1 to 1 ng/ml), synergised to establish an adjustable and sensitive NF-kB pathway response in hepatocytes. Higher LPS concentrations (> 5 ng/ml) induced sufficient TNFα amounts (> 0.3 ng/ml) from KCs or LSECs to induce a maximal NF-kB pathway response without any additive or synergistic effects. **Conclusion:** The combination of experimental data and computational multi-scale modelling illustrates that low doses of LPS and TNFα induce partial and adjustable cellular responses in non-parenchymal cells (LSECs and KCs) and hepatocytes. Under pathological conditions, high LPS/TNFα amounts stimulate non-adjustable and full-blown cellular responses. This integrative approach represents the basis for further analysis on the role of different cell types during inflammation-induced hepatic disease.

1.5 A disintegrin and metalloprotease 10 (ADAM10) is a central regulator of liver tissue homeostasis

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A Disintegrin And Metalloprotease (ADAM) 10 exerts essential roles during organ development and tissue integrity in many organs. However, only little is known about its implication in liver tissue physiology. We therefore generated mice deficient for Adam10 in hepatocytes, cholangiocytes and liver progenitor cells. While biliary tree formation and bile canaliculi morphology was normal in ADAM10Δhep/Δch animals, we observed areas with hepatocyte necrosis. This correlated with an impaired expression of bile acid transporters. Decreased numbers of necrotic areas in 15-week old ADAM10Δhep/Δch mice were clear evidence for on-going regenerative processes in ADAM10-deficient livers. Interestingly, we observed a strongly augmented ductular reaction that was accompanied by activation of hepatic stellate cells in 15-week old ADAM10Δhep/Δch mice, resulting in fulminant liver fibrosis. We show in cell-based assays that lack of ADAM10 in liver progenitor cells leads to enhanced c-Met signalling. In contrast to its role in other tissues, ADAM10 is dispensable for the Notch2-dependent biliary tree formation. However, our data demonstrate that ADAM10 is a central regulator of murine liver tissue homeostasis by regulating bile acid transporter expression and liver progenitor cell activation. As aberrant activation of liver progenitor cells is often found in chronic liver disease, ADAM10 may be considered a potential pharmaceutical target for the treatment of chronic liver disease.

1.6 A human hepatic in vitro co-culture system for the analysis of DILI related signaling

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The interaction between immune cells and hepatocytes during a sterile inflammation has come into focus in Drug-Induced Liver Injury (DILI) research during recent years. Therefore, this crosstalk was analysed in a novel indirect co-culture system with HepG2 and differentiated THP-1 cells using the antifungal drug ketoconazole as a known hepatotoxic model compound. The metabolism of ketoconazole, changes in the proteome, cytokine expression and secretion upon treatment in single- and co-culture were analysed. HepG2 metabolised ketoconazole to several metabolites which differed from those found in THP-1 cells both in single and co-culture. In the supernatant, the ketoconazole concentration decreased time-dependently while the concentration of several metabolites increased. The global proteomics analysis of HepG2 cells in co-culture identified the activation of the "Nrf2 mediated stress response" and the "Integrin linked kinase signalling" pathways after treatment with ketoconazole. Further upregulated proteins belonged to the NFκB, CXCL8 and sterol pathway. ELISA and qPCR assays revealed the upregulation of several pro-inflammatory cytokines including CXCL8, TNF-α and CCL3 in treated, co-cultured but not single-cultured HepG2 cells. The advantage of this indirect system with inserts is that both cell lines can be separated and analysed individually after co-culture and the results can be compared to single-cultured cells. The activation of signalling pathways related to the hepatotoxicity of ketoconazole occurred in the co-culture at lower concentrations, compared to the single-culture. In conclusion, the novel indirect co-culture system represents a promising new tool to study hepatotoxic drug effects in vitro.

1.7 A new mouse model of sclerosing cholangitis combining toxic and immune mediated bile duct injury

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Introduction: Primary Sclerosing Cholangitis (PSC) is a chronic cholestatic liver disease leading to fibrosis and obliteration of bile ducts, and eventually to cirrhosis and end stage liver disease. Currently there is no medical treatment with a proven benefit on disease progression. The etiology of PSC is largely unknown, but immune dysregulation and toxic biliary damage are considered to be part of PSC pathogenesis. There is an urgent need for a reliable mouse model, which allows a standardized testing of novel treatment strategies for PSC. We here describe a new mouse model of sclerosing cholangitis, which combines the two proposed mechanisms of PSC pathogenesis, toxic and immune mediated injury to bile ducts. **Methods:** B6-Mdr2ko mice, an established model of sclerosing cholangitis, were crossed with K14-OVAp mice, a model of acute, antigen driven cholangitis. Adoptive transfer of antigen specific OT-1 CD8 T cells in Mdr2xK14-OVAp animals was performed. On day 8 after adoptive OT-1 T cell transfer flow cytometry, histology, immunohistochemistry, ELISA, qPCR analyses and measurement of serum transaminase levels were performed. **Results:** The transfer of antigen specific OT-1 CD8+ T cells resulted in severe portal inflammation in recipient mice. Serum transaminase levels were significantly increased in OT-1 recipient mice in comparison to PBS control animals [PBS group (386 U/l) vs. OT-1 group (849 U/l) ($P = 0.0033$)]. Histopathologic examinations showed necrotic areas and increased lymphocytic infiltrations in mice after OT-1 CD8 T cell transfer, confirmed by elevated mHAI scores. Transferred OT-1 CD8+ T cells were mainly recruited to the liver of recipient mice and immunohistochemical staining confirmed congenic OT-1 CD8 cells within portal tracts and around biliary epithelial cells. Hepatic liver infiltrating T cells isolated from livers of OT-1 recipient mice produced siginificantly increased proinflammatory cytokine levels compared to cells isolated from PBS treated controls [IFNγ: PBS group (3015 pg/ml) vs. OT-1 group (8310 pg/ml) ($P = 0.0116$), TNFα: PBS group (351 pg/ml) vs. OT-1 group (679 pg/ml) ($P = 0.0344$)]. Moreover, T cell recruiting chemokines CXCL9 and CXCL10 as well as recruitment markers for proinflammatory lymphocytes, VCAM1 and ICAM1 were increased in OT-1 recipient mice. **Conclusion:** We here describe a new mouse model of PSC, which provides the opportunity to investigate the interaction between toxic and immunological injury of bile ducts and therefore better resembles the human situation than currently available models. This robust model can serve to test novel treatment strategies for PSC.

1.8 Beyond fibrosis: stellate cells as liver stem cells

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Research on hepatic stellate cells (HSC) largely focusses on their contribution to fibrogenesis in chronic liver diseases, but effective therapeutic strategies for patients are still not available. Knowledge about the relevance of HSC for normal liver homeostasis may facilitate the development of approaches to attenuate fibrosis and to support regeneration of injured liver. The expression of stem cell markers by HSC and their potential to undergo developmental processes indicate that functions beyond their contribution to extracellular matrix depostion and wound healing exist. Stellate cells possess an expression profile and differentiation potential related to bone marrow mesenchymal stem cells (bmMSC) and support hematopoiesis of hematopoietic stem cells. These typical features of bmMSC indicate that HSC represent liver-resident MSC. This classification is further supported by engraftment of transplanted HSC not only in the liver but also in the bone marrow of host animals. Transplantation and cell lineage-tracing studies in rodents further indicate that HSC can contribute to regeneration of injured liver by forming bile duct cells, hepatocytes and mesenchymal tissue. Collectively, the data

available thus far indicate a dual role of stellate cells in the liver by supporting stem/progenitor cells such as hematopoietic stem cells and to form new cell types through differentiation. The ultimate behavior of stellate cells is presumably context-dependent and controlled by internal or external signals. Bile acids are such environmental signals, since they are not only involved in metabolism but also in cell signaling. After liver injury, elevated levels of bile acids are detectable in the blood serum and can promote liver regeneration, while exceptionally high concentrations exert adverse effects. Interestingly, when isolated HSC and bmMSC from various species are exposed to low amounts of bile acids (e.g. 2 µM) such as tauroursodeoxycholic acid (TUDCA), their differentiation into hepatocytes is initiated. The TUDCA-mediated hepatic differentiation of MSC-populations involves the bile acid receptors farnesoid X receptor (Fxr) and transmembrane G-protein-coupled bile acid receptor 1 (Tgr5), but requires also supportive activity of notch, hedgehog and transforming growth factor-β family signaling. In contrast to this, β-catenin-dependent canonical Wnt signaling known to counteract non-canonical Wnt cascades inhibits TUDCA-initiated hepatic differentiation and represents a mechanism to avoid unneccessary cell differentiation in normal liver. In conclusion, bile acids represent important signaling molecules during liver regeneration, which can promote hepatic differentiation of adult stem cells such as MSC. Multipotent MSC occur in all organs and are represented by stellate cells in the liver. Since the therapeutic potential of MSC from various sources has become obvious in recent years, research on stellate cells as mediators of liver regeneration should be addressed by future research.

1.9 Cholestasis induces expression of the oncofetal marker Nope in adult murine liver independent of Fxr

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Background: Neighbor of Punc E11 (Nope) is an axonal guidance receptor that is strongly expressed in fetal and adult hepatic stem/progenitor cells and in hepatocellular carcinoma but not in terminally differentiated hepatocytes. We here investigated the expression pattern of the oncofetal marker Nope in adult mice in regenerating liver after biliary liver injury. **Methods:** Liver tissue was extracted from adult C57Bl/6 mice and Fxr-/- mice 24 hours up to 4 weeks after bile duct ligation (BDL) or after a 1 week diet containing cholic (0.5%) or ursodeoxycholic acid (0.5%). Liver tissue was tested for expression levels of Nope via quantitative RT-PCR. Costainings were performed for Nope in combination with CK19 (biliary), E-cadherin (epithelial) or the canalicular marker dipeptidylpeptidase (DPP) IV. For in vitro experiments, primary isolated murine hepatocytes were incubated with the bile acids TC, TLC and TDC (25 µM) for up to 5 days and expression of Nope was quantitatively analysed by RT-PCR. **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, $p < 0.001$). Costainings with E-cadherin and DPPIV demonstrate a sinusoidal expression pattern of Nope on hepatocytes, but no expression on CK19-positive cholangiocytes. At later stages after BDL, almost all of the hepatocytes stain positive for Nope. In Fxr-/- mice, Nope is expressed without additional biliary injury (28-fold vs. adult liver, $p < 0.0001$) and shows a further and significant increase after BDL (440-fold vs. no BDL, $p = 0.03$). A diet with cholic (26-fold vs. adult liver, $p = 0.002$) or ursodeoxycholic acid (8-fold vs. adult liver, $p = 0.004$) also leads to a significant expression of Nope with a membranous staining pattern on hepatocytes and a diet with hydrophobic acids results in a significantly higher expression of Nope ($p = 0.04$). In vitro, however, expression of Nope is increasing until day 5 independent of the presence of bile acids in the medium. **Conclusion:** We here report the bile acid-induced expression of the oncofetal marker Nope on (in vivo) adult hepatocytes probably indicating their dedifferentiation. The induction of Nope is not mediated through Fxr but lack of the receptor leads to a higher expression level probably due to limited compensatory mechanisms of bile acid homeostasis in hepatocytes and cholangiocytes.

1.10 Crucial role of the interplay of the MAPKAP kinases 2 and 3 for LPS-induced inflammation and their relevance for liver pathogenesis

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The liver plays a pivotal role in innate and adaptive immunity as it is frequently exposed to pathogens reaching the liver via the blood flow. It is important for the production of acute phase proteins, among others soluble pattern-recognition receptors and components of the complement system, which are important constituents of innate immunity. Moreover, the liver harbors the largest pool of resident tissue macrophages. They play a key role for the elimination of opsonized immune complexes by phagocytosis and they are important sources of inflammatory cytokines, which induce acute phase protein production in hepatocytes and further trigger innate immunity and subsequent formation of adaptive immunity. Within macrophages the MAPKAP kinases (MK)2 and 3, which represent downstream targets of the MAP kinase family member p38MAPK, are essential for the coordination of the inflammatory outcome as they regulate the expression of cytokines and type I IFN in response to viral as well as bacterial components in particular in the context of lipopolysaccharide (LPS), where MK2 and MK3 have been suggested to act on the one hand in a co-operative manner, as the expression of cytokines like TNF-α, IL-6 and IL-10 is abrogated upon deletion of MK2 and further diminished by the additional deletion of MK3. However, on the other hand MK2 and MK3 are also able to exert rather distinct than co-operative regulatory effects on gene expression as shown for IFN-β. According to our hypothesis, these distinct regulatory effects of MK2 and MK3 exclusively occur at the level of transcription, where MK2 controls gene expression by neutralizing inhibitory effects of MK3. Contrariwise, the co-operative regulation of gene expression by MK2 and MK3 occurs at the level of transcript stability. In our study we analysed LPS-induced gene expression in macrophages derived from wild-type animals as well as from animals deficient for MK2 or MK2 and MK3 using "whole genome microarrays". Our data suggest that there is a larger group of genes, which are controlled by MK2 and MK3 in a way that is comparable to that of IFN-β. Furthermore, we identified six different mechanisms of regulation and involved genes, which are controlled by these two MAPKAP kinases. Interestingly, this study further reveals that the deletion of MK2 or of both MK2 and MK3 results in massive alterations of the inflammatory response of macrophages towards LPS, which also includes that depending on the incubation period approximately 15 to 20% of genes are only regulated by LPS, when MK2 or MK2 and MK3 are absent. This indicates that apart from being critically involved in positive or negative regulation of gene expression in response to LPS MK2 and MK3 are also required to prevent a larger group of genes from being regulated in response to LPS. Understanding the molecular mechanisms of pathogen-induced gene expression in macrophages is important as they propose new potential targets for therapies against liver diseases. Our observations reveal that the interplay of MK2 and MK3 control a large subset of LPS-induced genes, which also involve newly identified target genes of MK2 and MK3.

1.11 Das synthetische Chaperon 4-PBA induziert eine Akut-Phase-Reaktion im Maus-Modell für Protein-Speicherkrankheiten

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Einleitung: Im HBs-transgenen Maus-Modell kommt es zur Akkumulation der HBV-Hüllproteine (HBs) im endoplasmatischen Retikulum der Hepatozyten. Die dadurch verursachte Ansammlung fehlgefalteter Proteine führt zu ER-Stress, auf den die Zellen mit der Unfolded Protein Response reagieren. Da der ER-Stress sowie die Akkumulation fehlgefalteter Proteine für einen Teil der Leberpathologie bei chronischer Hepatitis B verantwortlich gemacht werden, war es Ziel des Projekts den ER-Stress in den Leberzellen durch eine Behandlung mit dem synthetischen Chaperon 4-PBA (4-Phenylbutyrat) zu vermindern. **Methoden:** HBs-transgenen

Mäuse auf BALB/c Hintergrund und die HBs transgene murine Hepatozyten-Zelllinie AML-12 wurden mit 4-PBA behandelt. Hepatisches Gewebe und Zelllysate wurden mittels Microarray, quantitativer Real-Time-PCR und Western Blot Analyse analysiert. **Ergebnisse:** Die Hypothese, dass 4-PBA den ER-Stress im HBs-transgenen Maus-Modell vermindert, konnte nicht bestätigt werden. Die Microarray Daten vermitteln einen Anstieg von Akute-Phase-Proteinen wie beispielsweise Lipocalin 2 und Serumamyloid A (SAA). Dieser Anstieg konnte auf mRNA Ebene im Maus-Modell sowie in der Hepatozytenzelllinie bestätigt werden. Außerdem steigt die Genexpression bestimmter Cytokine (CCL-3, CCl-4, MCP-1, CXCL-1) im Maus-Modell an. In Western Blot Analysen konnte in einigen mit PBA behandelten Mäusen eine starke Lipocalin 2 Expression nachgewiesen werden. **Schlussfolgerung:** Es konnte gezeigt werden, dass das synthetische Chaperon 4-PBA eine Akute-Phase-Antwort in Mauslebern und Leberzelllinien verursacht. Offen ist noch, an welcher Stelle PBA ausschlaggebend eingreift. Unsere Ergebnisse legen nahe, dass Lipocalin 2 dabei eine wichtige Rolle spielt.

1.12 Deletion of WISP1 leads to higher sensitivity to carbon tetrachloride-induced liver damage

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WISP1 (Wnt-induced secreted protein-1) is a member of the CCN family of matricellular proteins. These highly conserved and secreted proteins can interact with integrins and modulate many biological processes like migration, proliferation, differentiation and apoptosis. CCN proteins have been show to play divergent roles in liver pathophysiology, in spite of having very similar structural domains. Importantly, little is known about WISP1 in this context. Gene array analysis of mouse liver after acute injury by intraperitoneal injection of CCl4 in C56BL/6N mice showed WISP1 mRNA upregulated during early injury phase, between 2 h and 24 h after CCl4 injection. These results were confirmed by real time PCR, showing a maximal induction of 40 fold over healthy liver at 24 h. To establish the role of WISP1 in acute liver damage we used a knockout mouse model, in which WISP1 expression is disrupted by replacing Exon 2 with a resistance cassette and a stop codon. Heterozygous mice were bred to establish homozygous knockout and wild type lines which were validated by genotyping. Real time PCR analysis confirmed the absence of WISP1 mRNA expression in KO mice challenged with CCl4. Remarkably, deletion of WISP1 led to a significant increase of necrotic area at 24 h. This was show by hematoxylin & eosin staining reaching 49% in KO vs. 27% in WT mice ($p < 0.0001$; $n = 13$ wild type, $n = 11$ KO) and serum transaminase activity (GPT: 8230 U/l in WT, 12300 U/l in KO). Importantly, immunofluorescence and real time PCR showed that WT and WISP1 KO mice have no significant differences in CYP2E1 expression, suggesting that the difference in tissue injury does not depend on alterations of CCl4 metabolism in KO mice. To further characterize this different phenotype, we performed a time course after CCl4 intoxication (460 mg/kg, i. p.) by collecting liver tissue at 30 min, 2 h, 8 h, 18 h, and 24 h. Interestingly, already at 18 h KO mice showed a clear necrotic area around the central veins whereas WT mice showed ballooned hepatocytes with cytoplasmic translocation of high-mobility group protein B1 (HGMB1), indicating that WISP1 KO mice had a more advanced stage of liver injury at this time point than the WT counterparts. This phenotype was also observed upon intoxication with lower doses of CCl4 (132.4 mg/kg and 76 mg/kg). Analysis of cytokine expression by real time PCR showed a stronger induction of TNFα in WISP1 KO mice (7-fold v/s 10-fold at 18 h, and 3-fold vs. 6-fold at 24 h in WT vs. WISP1 KO respectively), and IL6 (5-fold vs. 10-fold at 18 h, and no induction vs. 3-fold at 24 h in WT v/s WISP1 KO respectively). Furthermore, western blot analysis showed marked alterations in signal transduction pathways in liver tissue of WISP1 KO mice, with stronger activations of p-JNK at 2 h and p-STAT3 at 18 h and 24 h. Currently we are investigating the mechanism through which WISP1 exerts a protective role upon CCl4 stimulation in liver damage and in the subsequent regeneration process. In conclusion we have strong evidence that WISP1 plays an important role in acute liver damage.

1.13 Delta like ligand4 modulates chemokine ligand 2 through impacting the NFkB pathway

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Introduction: Our previous study showed that recombinant Delta like ligand 4 (rDll4) attenuated liver damage in carbon tetrachloride challenged mice through inhibiting chemokines, particularly chemokine ligand 2 (Ccl2). This study investigated the role of rDll4 in mice with bile duct ligation (BDL) and mechanisms how Dll4 may regulate Ccl2. **Methods:** The function of Dll4 was examined in BDL-challenged mice. The effect of Dll4 on LPS-induced-Ccl2 expression was investigated in macrophages (RAW264.7 cells) using qPCR, immunoblot, and Ccl2 promoter reporter assays. **Results:** In BDL mice, rDll4 induced massive hepatic necrosis, resulting in the death of all animals within one week. Compared to control BDL mice, inflammatory cell infiltration and expression of Ccl2 were significantly reduced in rDll4-receiving BDL mice. Recombinant Ccl2 application rescued BDL mice from rDll4-mediated death. In RAW264.7 cells, rDll4 inhibited LPS-induced Ccl2 expression and LPS-induced p65 nuclear translocation, a key event of NFkB signalling pathway activation. Administration of JSH-23, a specific NFkb inhibitor, completely blocked LPS-induced expression of Ccl2. **Conclusion:** Dll4 modulates liver inflammatory response by downregulating Ccl2 expression, which is probably mediated by a link between Notch and NFkB signalling pathways.

1.14 Die Hemmung der Glyoxalase-I durch Ethylpyruvat vermindert den LPS-induzierten Anstieg des portalen Drucks

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Einleitung: LPS führt über Bildung von Methylglyoxal (MGO) und advanced glycation endproducts (AGEs) zur Freisetzung proinflammatorischer Zytokine. MGO wird durch die Glyoxalase-I (Glo-I) enzymatisch detoxifiziert. Die Hemmung der Glo-I durch Ethylpyruvat (EP) zeigt antiinflammatorische Effekte. Ziel der Arbeit war die Analyse von Glo-I in sinusoidalen Endothelzellen und dessen Einfluss auf den portalen Druck. **Methode:** In der TSEC-Endothelzelllinie erfolgte mittels Enzymkinetikmessung, RT-PCR und Western-Blot die Bestimmung der Aktivität und der Expression der Glo-I. TSEC wurden über 24 h mit 100 ng/ml LPS ± 1 – 20 mM EP inkubiert, es erfolgte die Analyse von eNOS/peNOS sowie Akt/pAkt im Western-Blot. Wistar-Ratten ($n = 30$) wurden i. p. mit LPS (1 mg/kg KG) ± EP (40 mg/kg KG) oder NaCl behandelt, nach 4 h erfolgte die Messung des portalen Drucks (PVP). **Ergebnisse:** Glo-I konnte in TSEC nachgewiesen werden. Die Behandlung von TSEC mit LPS über 24 h führte zum signifikanten Anstieg von peNOS (172,5 ± 20 vs. 100 ± 3%, $p = 0,02$) und pAkt (161,8 ± 9 vs. 100,0 ± 9%, $p = 0,01$). Die Gabe von LPS und 20mM EP reduzierte den LPS-induzierten Anstieg von peNOS (22,2 ± 3%, $p = 0,002$) und pAkt (25,0 ± 8%, $p < 0,001$). Die Gabe von LPS führte zum sigifikanten Anstieg des PVP (mm Hg) von 9,0 ± 0,4 auf 11,2 ± 0,6 ($p = 0,03$), die zusätzliche Gabe von EP ergab einen Abfall des PVP auf 8,9 ± 0,3 ($p = 0,01$). **Schlussfolgerung:** Ethylpyruvat vermindert die LPS-induzierten Anstieg des portalen Drucks. Die Effekte werden über Glo-I und eine verminderte Phosphorylierung von eNOS und Akt vermittelt. Glo-I könnte daher einen neuen Mechanismus in der Aktivierung von sinusoidalen Endothelzellen und inflammatorisch bedingter Erhöhung des portalen Drucks darstellen.

1.15 Die Repression von miR-192 schützt vor hepatischer Ischämie/Reperfusionsschädigung

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Einleitung: miRNAs sind in die hepatische Genexpression unter physiologischen und pathophysiologischen Bedingungen involviert. Neben einer Rolle als Regulatoren der Genexpression können miRNAs auch als Biomarker genutzt werden, in diesem Zusammenhang konnten wir kürzlich zeigen, dass erhöhte Serumspiegel der leberspezifischen microRNA miR-122 spezifisch mit dem Vorliegen eines Leberschadens bzw. Leberzelluntergang korrelieren. Die Rolle der miR-192 im akuten Leberschaden ist bislang nicht untersucht worden. **Methodik und Resultate:** Zur Untersuchung der Rolle der miR-192 im akuten Leberschaden wurde zunächst ein hepatischer Ischämie/Reperfusionsschaden (I/R) in der Maus induziert. Es zeigten sich deutlich erhöhte miR-192-Serumspiegel. Diese korrelierten mit Markern des akuten Leberzellschadens (ALT/AST; miR-122 Serumspiegeln) und dem Ausmaß des Leberzelltodes (Anzahl TUNEL positiver Zellen). Zahlreiche Autoren konnten in der Vergangenheit zeigen, dass Veränderungen der miRNA-Serumkonzentrationen spezifisch Veränderungen der intrazellulären miRNA-Expression anzeigen und somit zelluläre Adaptationsprozesse reflektieren. Wir untersuchten daher in einem nächsten Schritt die intrahepatische miR-192 Expression nach I/R. Hier zeigte sich bemerkenswerterweise eine Minderexpression der miR-192. Diese Regulation war auf die Hepatozyten beschränkt, während in Immunzellen die miR-192 Expression unverändert blieb, was auf eine Spezifität des Effektes hindeutet. Eine Minderexpression der miR-192 konnte auch in einem in vitro Modell der I/R beobachtet werden (H2O2-Behandlung von Hepatomzellen). In diesem Modell führte die Überexpression der miR-192 führte zu einem vermehrten Zelluntergang, so dass die Minderexpression der miR-192 protektiv wirkt. Dieser Effekt konnte auf eine Derepression antiapoptotischer Gene (z. B. Zeb2) zurückgeführt werden. **Schlussfolgerung:** Die vorliegenden Daten legen eine Rolle der miR-192 in der Pathophysiologie akuter Lebererkrankungen nahe.

1.16 Differential activation of RhoA and c-SRC in experimental and human fibrosis

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Introduction: RhoA orchestrates cytoskeleton formation, migration and mobility via c-SRC and mDia1 in different cells. RhoA/ROCK also plays a crucial role in hepatic stellate cells and hepatic fibrogenesis. Matrix stiffness promotes HSC activation via cytoskeleton modulation. The role of SRC and mDia1 in HSC, and the role of matrix stiffness, were investigated in this study. **Methods:** Liver fibrosis was induced in rats using BDL, TAA or CCl4 models. Samples from cirrhotic patients and controls were collected at liver transplantations and tumor resections. Western Blot using phospho-specific antibodies against active p-c-SRC-Y418 and inactivated p-c-SRC-Y530 was performed. LX2-cells were cultured on acrylamide gels of various elasticities to simulate non-fibrotic or fibrotic environments, then exposed to SRC-inhibitor PP2. RNA levels of RhoA, mDia, Col1A1 and α-SMA were analyzed via qRT-PCR. **Results:** RhoA expression was significantly elevated in human and experimental liver fibrosis. While c-SRC expression remained unchanged, increased p-c-SRC-Y530 (inactive form) was observed in fibrotic liver samples, and p-C-SRC-Y418 (active form) was markedly reduced in Western Blot. Similarly, expression of mDia1 was reduced in liver fibrosis. mRNA levels of RhoA and Albumin were reduced in hepatocytes that were cultured on PAA gels with low elasticity. In LX-2 cells cultured in a stiff environment, mRNA levels of RhoA, Col1A1 and α-SMA were significantly increased. Inhibition of c-SRC in LX2-cells lead to an increase in Col1A, α-SMA and mDia most prominenly in the stiff environment. **Conclusions:** In this work we investigated the role of the expression and interaction of these pathways in liver fibrosis. We suggest that c-SRC is inactivated in liver fibrosis by phosphorylation, and that inactivation of c-SRC is crucial for cytoskeletal regulation in liver fibrogenesis.

1.17 Ein zellspezifisches Netzwerk TGF-beta abhängiger micro-RNAs reguliert organübergreifende Prozesse in der Fibrogenese

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Einleitung: miRNAs stellen eine neue Regulationsebene transkriptioneller Prozesse dar. Es gibt Hinweise, dass organübergreifende miRNA-abhängige Regulationsmechanismen konserviert sein könnten. In der aktuellen Studie sollte daher eine systemübergreifende Analyse in verschiedenen Modellen der Organfibrose durchgeführt werden, um Netzwerke von miRNAs zu identifizieren, die organübergreifend die Fibrogenese kontrollieren. **Methodik:** Die Expression von miRNAs wurde mittels Array- und PCR-basierter Techniken in murinen Modellen der Organfibrose (Leber, Niere, Lunge) gemessen und mit Messungen an Patientenproben korreliert. Darüber hinaus wurden Zellseparationstechniken verwendet und mittels Stimulations- und Transfektionsansätzen die Funktion dieser miRNA analysiert. **Resultate:** Fünf microRNAs (miR-29c, miR-30c, miR-193 und mir-199a-3 p/-5) zeigten eine konkordante Dysregulation in allen untersuchten Fibrosemodellen. Eine Rolle in der Leberfibrose war für drei dieser microRNAs (miR-29c, miR-199a-3 p/-5 p) publiziert worden. In den folgenden Untersuchungen wurde daher der Fokus auf die miR-30c und miR-193 gelegt. Die in der Arrayanalyse beobachtete Minderexpression der miR-30c bzw. miR-193 wurde mittels PCR sowohl in den unterschiedlichen Organfibrosen als auch in den Modellen (CCl4-Injektion, bile duct ligation) der murinen Leberfibrose sowie in korrespondierenden humanen Proben verifiziert. Auf zellulärer Ebene konnte gezeigt werden, dass die Fibrose-assoziierte Minderexpression dieser miRNAs auf die hepatischen Sternzellen beschränkt war und durch TGF-beta abhängige Mechanismen hervorgerufen wurde. Auf funktioneller Ebene zeigte sich, dass eine Minderexpression der miR-30c bzw. der miR-193 zu einer verstärkten Aktivierung und einem profibrotischen Phänotyp von HSC führen. Dies konnte auf die Regulation eines Netzwerks von fibroseassoziierten Genen und insbesondere ECM-Genen (TGF-beta, SNAIL) durch diese miRNAs zurückgeführt werden. **Schlussfolgerung:** Die Dysregulation bestimmter „Fibrose-miRs" stellt ein organübergreifendes Phänomen in der Fibrogenese statt.

1.18 Epigenetic changes contribute to hepatic stellate cell activation

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Introduction: Hepatic stellate cells (HSC) were recently identified as mesenchymal stem cells of the liver that exhibit a remarkable differentiation potential. The differentiation is preceded by an activation process. The aim of this study is to characterize the dynamics of DNA methylation during HSC activation to provide information about the epigenetic regulation of adult stem cells. **Methods:** Primary rat HSC were activated in cell culture within 7 days. DNA methylation of quiescent and activated HSC was determined using ELISA and immunofluorescence with 5mC-specific antibodies for global as well as reduced representation bisulfite sequencing (RRBS) for genome-wide DNA methylation. Examination of gene expression was performed with mRNA microarrays and quantitative real time PCR. Protein amount was determined by Western blot. **Results:** Global DNA methylation decreased by 60% during HSC activation, while surprisingly, RRBS data revealed that differentially methylated regions (DMR) in promoter and intragenic regions got rather hyper- than hypomethylated at the same time. The existence of DMRs coincided with expression changes of associated genes. Gene ontology (GO) term analysis showed that differentially methylated genes clustered for cellular processes like cell activation and differentiation. Furthermore, genes involved in Wnt signaling and developmental genes, like Lhx6 or Klf2 ex-

hibited differential methylation and altered gene expression during HSC activation. Inhibition of the cell cycle by L-mimosine treatment revealed that the DNA demethylation is independent of replication, indicating active DNA demethylation processes. In addition, the amount of factors associated with active DNA demethylation increased during activation, which further strengthens this idea. **Conclusion:** HSC run through substantial epigenetic changes during activation. It will be interesting to further unravel the contribution of epigenetics to HSC activation and also differentiation to gain more insight into adult stem cell regulation.

1.19 Erhöhte Mortalität durch leberspezifische Koexpression der profibrogenetischen Faktoren PDGF-B und TGF-b

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Leberspezifische Überexpression des profibrogenetischer Faktors PDGF-B induziert die Ausbildung einer Leberfibrose. Des weiteren konnte gezeigt werden, dass die leberspezifische Überexpression von PDGF-B auch eine erhöhte Expression von TGF-b und des TGF-b Rezeptors Typ II induziert. Allerdings induziert TGF-b auch die Expression von PDGF-B. Um die Kooperativen Effekte einer PDGF-B und TGF-b Überexpression zu untersuchen, haben wir PDGF-B und TGF-b hepatozytenspezifisch doppeltransgene Tiere im Vergleich zu jeweils einzelntransgenen und Wildtyptieren untersucht. Während die PDGF-B transgenen Tiere PDGF-B konstitutiv überexprimieren wird bei den TGF-b transgenen Tiere die TGF-b Überexpression mittels LPS konditionell ab einem Alter von 4 Wochen induziert, um die kooperativen Effekte von TGF-b bei einer bereits vorhandenen PDGF-B Überexpression zu analysieren. Dabei wurden alle Versuchstiere parallel mit LPS behandelt, um auch die Effekte der LPS-Applikation zu berücksichtigen. Beobachtung der Tiere erfolgte bis zu einem Alter von 24 Wochen. Bis zu einem Zeitpunkt von 20 Wochen versterben alle PDGF-B/TGF-b doppeltransgenen Tiere, wohingegen die Wildtyp- als auch die PDGF-B einfachtransgenen Tieren überleben. Die TGF-b einfachtransgenen Tiere versterben lediglich zu 20%. Im weiteren wurden Tiere im Alter von 12 Wochen untersucht, da zu diesem Zeitpunkt noch 60% der PDGF-B/TGF-b doppeltrangenen Tiere lebten. Diese Beobachtungen implizieren, dass eine TGF-b Überexpression in Kooperation mit einer PDGF-B Überexpression zu signifikanten physiologischen Veränderungen führt, welche nicht allein auf die Fibrogenese zurückzuführen sind. Initial wurden an Tieren der einzelnen Versuchsgruppen im Alter von 12 Wochen Genexpressionsanalysen mittels Microarrayhybridisierungen durchgeführt. Die hierarchische Clusternalyse der Microarraydaten zeigte, dass sich die Versuchsgruppen in erster Ebene über PDGF-B auftrennen und dann in zweiter Ebene es durch TGF-b zu einer jeweils klaren Auftrennung der jeweiligen Versuchsgruppen kommt. Eine Netzwerkanalyse der Versuchsgruppen zeigte, dass sich bei den PDGF-B/TGF-b doppeltransgenen Tieren neben zentralen Netzwerkpunkten wie z.B. Tnf, Akt oder p53 (die sich auch in den PDGF-B bzw. TGF-b einzeltransgenen Tieren finden lassen), sich mit Jun sich ein weiterer zentraler Netzwerkpunkt herauskristallisiert. Zusammenfassend zeigen unsere Ergebnisse, dass zwischen PDGF-B und TGF-b ein komplexes Zusammenspiel existiert, das von signifikanter physiologischer Bedeutung ist. Weiterführende Analysen sollen nun die molekularen Kernnetzwege und darin zentral regulatorische Gene identifizieren.

1.20 Expression, epigenetical regulation and signaling network of embryonic stem cell-expressed RAS in hepatic stellate cells

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Hepatic stellate cells (HSC) are liver-resident mesenchymal stem cells with progenitor characteristics. HSCs are activated during liver injury and are involved in pivotal processes e.g. liver development, immunoregulation, regeneration and also fibrosis [1,2]. To date, several controversial studies reported the candidate pathways that regulate the plasticity of HSC towards distinct physiological and pathophysiological processes. Here we analyzed the expressional changes and activity of small GTPases of the RAS family, and investigated the signaling networks of quiescent HSC versus activated HSC. For the first time, we report that embryonic stem cell-expressed RAS (ERAS) [3] is specifically expressed amongst the liver cells in quiescent primary HSCs and downregulated in the course of culture-induced HSC activation dues to promoter DNA methylation. Notably, the high level of the ERAS protein correlates with the activation of mTORC2, AKT, and HIPPO in quiescent HSCs. We showed that RAS signaling in activated HSC is more towards RAF-MEK-ERK pathway while quiescent HSC rely on the AKT activation, which may in turn result from an ERAS-derived activation of PI3K and mTORC2 pathways. These and other mechanisms controlling the HSC fate/function will be discussed. **References:** [1] Kordes, C. & Häussinger, D. Hepatic stem cell niches. Journal of Clinical Investigation 123, 1874 – 1880 (2013). [2] Kordes, C., Sawitza, I., Götze, S., Herebian, D. & Häussinger, D. Hepatic stellate cells contribute to progenitor cells and liver regeneration. Journal of Clinical Investigation 124, 5503 – 5515 (2014). [3] Nakhaei-Rad, S., Nakhaeizadeh, H., Kordes, C., Cirstea, I.C., Schmick, M., Dvorsky, R., Bastiaens, P.I., Haussinger, D. & Ahmadian, M.R. The function of embryonic stem cell-expressed Ras (E-Ras), a unique Ras family member, correlates with its additional motifs and its structural properties. J Biol Chem (2015).

1.21 FPR1 might play a relevant role in prevention of liver fibrosis

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Introduction: Formylpeptide receptors (FPR) are mainly studied as chemotactic receptors during sterile and pathogen associated inflammation. They are expressed on immune cells but also non-hematopoietic tissues. Theirs ligand fMLF is represented on bacteria or is secreted by mitochondria after apoptosis. Together they can induce a strong chemotactic movement. The role of FPRs in during hepatitis and liver fibrosis is so far poorly understood. Besides, immunoregulation and maintainance of immunehomeostasis is important to prevent hepatitis and to avoid the establishment of liver fibrosis. **Material and Methods:** We investigated the deficiency of FPR1 and FPR2 under the condition of the liver fibrosis inducing CCl4-model. Three groups of mice, Wildtype (WT), FPR1-knockout (F1KO) and FPR-2 knockout (F2KO) were treated with CCl4 for a period of 8 weeks. Afterwards serum and liver tissue was analyzed in more detail by qPCR and IHC. **Results:** After finalization of the CCl4 treatment WT and F2KO-mice displayed a higher gain of weight after 8 weeks compared to F1KO-mice. Analysis of transaminases in the serum of the diverse mice strains revealed no detectable differences in the ALT-levels after CCl4 treatment. The histological analysis displayed a clear fibrotic conversion in the livers of all genotypes. Investigation of collagen deposition in the liver by Sirius Red staining revealed a significant higher amount of collagen fibers detectable in the liver of F1KO-mice compared to WT- and F2KO-mice. Interestingly the expression of the Col1A1-gene was significantly higher in both FPR-KO-mice strains compared to WT-mice. The gene expression of collagen degrading enzymes such as MMP13 was highly altered. MMP13 was higher expressed in WT and F2KO compared to F1KO. Quantitative PCR analysis for the gene expression of pro-inflammatory cytokines such as IL-6 and TNF-α displayed a significant reduction of these two genes in F1KO mice in comparison to the other groups. Contradictory to this, CD45+-cells were stronger represented in livers of FPR1-KO mice compared to WT and F2KO-mice. Also the liver regeneration, detected by PCNA-IHC, was impaired in F1KO-mice compared to wildtype and F2KO-mice. **Discussion:** The deficiency of Formylpeptide receptors leads to various effects in the liver of the knockout mice. Whereas lack of FPR2 seems to have a relatively mild effect on liver fibrogenesis and liver inflammation, FPR1 appears to be more relevant in the regulation of liver inflammation and liver regeneration. Loss of FPR1 was associated with an increased hepatitis and decreased liver regeneration compared to WT-mice. However, the mechanism behind the observed phenomenon remains to be further investigated and deciphered.

1.22 Hepatic Proteome and Lipid Profiling of Wild Type and Lipocalin-2-Deficient Mice in Experimental Steatosis

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Background: Lipocalin-2 (LCN2) or neutrophil gelatinase-associated lipocalin (NGAL) is a small secreted adipokine belonging to the lipocalin family [1]. It binds and transports small hydrophobic molecules and limits bacterial growth by sequestering iron-containing siderophores. In the liver, LCN2 plays a protective role in inflammation, infection, and cellular stress. Recently, we demonstrated that LCN2 regulates lipid droplet protein Perilipin 5 (PLIN5) expression in primary hepatocytes and showed that LCN2 animals are more prone to hepatic inflammation and steatosis [2 – 4]. **Methods:** We here comparatively analyzed the proteome (label-free proteomics or 2D-DIGE protein expression profiling) of wild type and Lcn2-deficient mice fed either a standard-chow and a methionine- and choline-deficient (MCD) diet. The differential expression was confirmed by Western blot analysis and quantitative real-time PCR. We further employed comparative MALDI-TOF Imaging Mass Spectrometry to monitor the spatial distribution of a broad range of lipids in liver tissue sections of respective groups. **Results:** We identified a multitude of genes that are either upregulated during hepatic steatosis or differentially induced or repressed in mice lacking LCN2. Differentially expressed proteins were BRIT1/MCPH1, FABP5, HMGB1, HBB2, and L-FABP. In addition, we identified significantly altered m/z signal intensities for several sphingomyelins, triglycerides, and phospholipid species. Most notably, phosphatidylinositol phosphates were substantially elevated in MCD-fed mice, indicating chronic activation of phosphatidylinositol phosphate-dependent signaling pathways, and this alteration was unaffected by LCN2 deletion. Moreover, the abundance of some 20:4 lipids were elevated in the livers of Lcn2-deficient mice suggesting that this gene disruption might interfere with arachidonic acid and eicosanoid metabolism. **Conclusion:** In summary, our data indicate that LCN2 is a key switch influencing triglyceride balance, reactive oxidative stress formation, inflammatory response, and cellular apoptosis. **References cited:** [1] Asimakopoulou A, Weiskirchen R. Lipocalin 2 in the pathogenesis of fatty liver disease and non-alcoholic steatohepatitis. Clinical Lipidol. 2015;10:47 – 67. [2] Borkham-Kamphorst E, van de Leur E, Zimmermann HW, Karlmark KR, Tihaa L, Haas U, Tacke F, Berger T, Mak TW, Weiskirchen R. Protective effects of lipocalin-2 (LCN2) in acute liver injury suggest a novel function in liver homeostasis. Biochim Biophys Acta 2013;1832:660 – 73. [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. [4] Asimakopoulou A, Borkham-Kamphorst E, Tacke F, Weiskirchen R. Lipocalin-2 (NGAL/LCN2), a "HELP-ME" signal in organ inflammation. Hepatology 2015; Jun 5 [Epub ahead of print]

1.23 Hepatocyte dependent induction of regulatory T-cell subsets

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Introduction: The liver is continuously exposed to exo- or endogenous antigens and toxins. Therefore, the liver is able to induce a tolerogenic environment. It is known that regulatory T cells have a pivotal role in inducing this immunotolerance. Several liver resident cells, like liver sinusoidal endothelial cells (LSECs) or Kupffer cells (KCs), are able to induce regulatory T cells. Investigations showed that T cells and hepatocytes, the most abundant cell type within liver, are able to communicate directly through fenestrations in the sinusoidal endothelium. Therefore, we studied if the interaction between hepatocytes and T-cells induce regulatory T-cell subsets. **Methods:** Splenic CD4+ T-cells from C57BL/6, FIR x tiger (foxp3-RFP and Il10-eGFP double reporter mice), DEREG (foxp3-eGFP reporter mice), Gzmb ko or CD45.1 congenic mice were purified via MACS sorting and co-cultured with hepatocytes isolated from C57BL/6 mice. Gene expression was measured by nCounter Gene Expression or PCR. Cytokine expression was detected via ELISA. Proliferation of eFluor670 labelled responder T cells was determined by flow cytometry. **Results:** Our results show that hepatocytes induce regulatory T cells which produce IL-10 as well as IFNγ and lack Foxp3 expression. Furthermore this hepatocyte induced T cells produce an increased amount of the serine protease Granzyme B. The Granzyme B, such as the IFNγ and IL10 expression are dependent on Notch signalling. The hepatocyte induced T cells show a high capability to suppress responder T cells in vitro, which is IL-10 independent. In presence of TGFβ hepatocytes induce CD25 +Foxp3+Tregs which are also able to suppress responder T cells in vitro. **Conclusion:** We demonstrated that hepatocytes induce IL-10+ and IL10-regulatory T cell subsets that inhibit activation and proliferation of naive CD4+ T cells in vitro thus, suppression occurs independently of IL-10. Without TGFβ in the culture the regulatory T cell subsets are characterized by high Granzyme B expression that is induced via the Notch pathway. As Granzyme B expression in regulatory T cells has been described as one way of suppression we currently perform experiments with Granzyme B knockout mice. Another possible suppressive mechanism we analyse is the depletion of IL-2 and downregulation of CD25 in responder T cells by hepatocyte induced T cells. To get further information about the function of these cell types in vivo, we establish adaptive transfer experiments with hepatocyte induced regulatory T-cell subsets in a mouse model of immune-mediated hepatitis.

1.24 Hypoxia and inflammation reduce the expression of the mineralocorticoid receptor (MR) in the hepatocytes – a mechanism that explains the lower expression of MR in cirrhosis

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Introduction: Hypoxia and inflammation are the main mechanisms in the development of cirrhosis. Cirrhosis leads to increased concentration of aldosterone. Aldosterone activates the MR. The aim was to investigate the role of MR during the development of cirrhosis. **Methods:** Fibrosis and cirrhosis was induced by CCl4 administration for 5, 8 or 12 weeks (w). Total liver tissue, hepatocytes (Hep), hepatic stellate cells (HSC) and endothelial cells (LSEC) were isolated. Gene expression was analyzed using qRT-PCR and droplet digital PCR. Hepatoma cells (MH1C1) were treated with cytokines (IL-1ß, IL-6 and TNF-α each 10 ng/ml) and hypoxia (0.1% O2) for 24 hours. **Results:** In normal livers copy number of MR mRNA was increased 10-fold in Hep (mean ± SEM: 9914 ± 2622 mRNA/µg RNA) compared to HSC (1027 ± 275; $p < 0.05$) and LSEC (1280 ± 120; $p < 0.05$). Developing of cirrhosis led to reduction of MR in Hep (8w: 52 ± 4.9%, $p < 0.05$; 12w: 6.0 ± 3.0%, $p < 0.01$) compared to normal (100%). Treatment of MH1C1 with cytokines alone did not change the expression of MR. Hypoxia alone leads to a decrease in MR mRNA (compared to normoxia: 22 ± 3.5%; $p < 0.01$) and protein (51 ± 5.6%; $p < 0.05$). Co-incubation using cytokines and hypoxia revealed a decrease of MR mRNA (13 ± 1.1%; $p < 0.01$) and protein (18 ± 2.9%; $p < 0.01$). **Conclusion:** MR is mainly expressed in Hepatocytes and less in HSC or LSEC. Expression of MR decreases during the development of cirrhosis in Hepatocytes. Hypoxia reduces the expression of MR in Hepatocytes. Combination of hypoxia and inflammation further decrease the expression of MR. These results might explain the decrease in MR expression in liver cirrhosis.

1.25 Identification of transcriptional regulatory networks in acute liver damage and regeneration

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Acute liver injury triggers a myriad of signaling and transcriptional events, including stress and inflammatory transcriptional regulatory networks (TRN). These pathways may exacerbate damage or promote regeneration, depending on factors such as timing, duration, intensity and crosstalk. To identify TRN induced early and late after hepatotoxic exposure, we analyzed mouse liver tissue after a single CCl4 administration (1.6 g/kg) using Affymetrix gene arrays and bioinformatics, combined with histological and western blot analyses, at early (2 h, 8 h), intermediate (1, 2, 4 days) and late time points (8 and 16 days). The time-resolved analysis revealed a highly dynamic gene expression response upon hepatotoxicity, with 1,469 genes deregulated ($p < 0.05$, FDR adjusted). Bioinformatics analyses allowed the identification of five time-dependent biological motifs. The first gene expression wave consisted of genes with maximal induction at 2 h with enrichment of MAPK-associated genes such as c-Jun and Fos. Western blot analyses confirmed the early activation of MAPK with ERK and JNK phosphorylation at 2 h. Two subsequent clusters, with maximal inductions at 8 and 24 h contained an overrepresentation of "protein processing in the endoplasmic reticulum" motifs, suggesting activation of ER-stress pathways. Analysis of Xbp1 splicing and induction of ER-stress markers such as the transcription factor CHOP and p-eIF2α confirmed activation of ER-stress between 2 h and day 1 after CCl4 intoxication. In parallel, we also observed a massive downregulation of metabolism related genes with maximal repression at 8 h and day 1. These clusters included p450 enzymes such as CYP2E1 and CYP3A4. Liver-enriched transcription factors were also downregulated including FXR, SHP and PPARA. Analysis of overrepresented transcription factor binding sites identified HNF1 and HNF4 in the downregulated gene clusters. The last two gene clusters, containing genes maximally upregulated at day 2 and day 3 represented the proliferation response that occurs after damage, including genes such as cyclins and cyclin-dependent kinases. Histological analyses of c-Jun and CHOP demonstrated that the initial burst of stress signaling occurs in pericentral regions. Interestingly, proliferation-associated genes such as c-Myc also show a pericentral expression, suggesting that stress and regeneration signaling propagate as a wave from the pericentral area. In contrast, the expression of HNF4a was downregulated in all liver parenchyma, suggesting that some TRN are zonally restricted while others occur in all hepatocytes. Inflammation-associated genes were observed in all gene clusters, including chemokines such as Cxcl1 (2 h), Ccl2 (8 h), and acute phase response genes such as Lcn2 (8 h). In conclusion, the basic blueprint of the TRN identified in this study will serve as an excellent basis to unveil the precise contribution of further signaling and transcriptional responses induced in acute liver injury.

1.26 Increased expression of histone deacetylase 7 during hepatic stellate cell activation promotes pro-fibrogenic gene expression

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Recent evidence has highlighted a pathological imbalance in hepatic fibrosis between the acetylation and deacetylation of histone proteins regulated by histone deacetylases (HDACs). However, the role of individual HDACs in liver fibrosis is almost completely unknown. Recently, we identified that HDAC7 can bind to the promotor of the hepatocyte growth factor (HGF) gene, and herewith, suppresses the expression of this anti-fibrogenic factor in activated hepatic stellate cells (Hepatology. 2014 Sep;60(3):1066 – 81). The aim of this study was to get further insight into the expression and function of HDAC7 in chronic liver disease. **Methods and Results:** HDAC7 expression was significantly increased in different murine models of chronic liver injury (bile duct ligation, thioacetamide intoxication and diet induced non-alcoholic steatohepatitis (NASH)) as well as in liver specimens from patients with NASH or liver cirrhosis of different origin compared to healthy control liver tissue. HDAC7 expression revealed a significant correlation with the expression of collagen type I as well as alpha-smooth muscle actin, a marker of hepatic stellate cell (HSC) expression. In line with this, HDAC7 mRNA and protein expression markedly increased during in vitro activation of murine as well as human HSCs. Stimulation with platelet-derived growth factor beta (PDGFB) further dose dependently induced HDAC7 expression in activated HSCs. Suppression of HDAC7 expression with si-RNA caused significantly reduced MMP10 expression in activated HSCs. In line with this, analysis of HDCA7 and MMP10 expression in activated HSCs from 14 different human donors showed a significant correlation. Moreover, HDCA7 expression correlated with collagen type I expression in these 14 HSCs. **Conclusion:** Increased HDAC7 expression during HSC activation causes induction of profibrogenic genes and may also at least in part explain PDGFB induced profibrogenic effects on activated HSCs. Together with our previous finding that HDAC7 suppresses anti-fibrogenic HGF expression these data indicate HDAC7 as critical orchestrator of the pathologically impaired histone (de)acetylation in liver fibrosis.

1.27 Influence of the microbiome in regulating ConA-induced-liver injury

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Introduction: The liver is continuously exposed to gut derived factors and bacteria. It has been shown, that gut microbiota might be involved in liver diseases such as non-alcoholic fatty liver disease, hepatic encephalopathy, as well as viral, and alcoholic hepatitis. Nevertheless, little is known about the influence of gut microbiota on immune-mediated liver injury. The role of gut microbiota in Concanavalin A (ConA)-induced T-cell mediated hepatitis was investigated in antibiotic treated mice. **Materials and Methods:** Mice were treated with antimicrobial drugs (Neomycin, Bacitracin, Pimaricin) for 8 days prior to ConA challenge. Liver injury induced by ConA was assessed by determination of serum ALT activities. Immunophenotyping of infiltrating cells was performed via flow cytometry. Feces of mice treated with antibiotics was collected and will be analyzed for the microbial composition by 16S rRNA sequencing. **Results:** Treatment with the broad-spectrum antimicrobial cocktail significantly ameliorated ConA-induced liver damage. Frequencies of T cell populations including regulatory T cells were unaltered. Moreover, the protective effect did not correlate with reduced expression of IFNγ or FasL. Interestingly, CCR9+ cells within the CD11bhighCD11c- cell population were significantly elevated. This increase was also detectable in the (F4/80+) and antigen- presenting (MHCII+) subpopulation of CD11bhighCD11c-cells. **Conclusion:** We clearly demonstrated a correlation between gut microbiota and ConA-induced liver damage. The antimicrobial drugs induced higher frequencies of CCR9+ CD11bhigh CD11c-activated macrophages, possibly due to the absence of endotoxin, which is known to induce an inhibitory phenotype of liver macrophages. Future experiments are intended to identify immune-regulatory cell populations in the liver. In addition, we will analyze the composition of the gut microbiota in the feces and will correlate these results with the disease severity and immune response.

1.28 Lipocalin 2 und Perilipin 5 in der Pathogenese der durch Fruktose ausgelösten NAFLD

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Hintergrund: Die nicht-alkoholische Fettlebererkrankung (NAFLD) wird von hepatischer Insulinresistenz und Leberverfettung begleitet [1,2]. Die Zunahme an Fettsäuren in Hepatozyten steigert die Expression von Zytokinen und führt zur Entzündung. Übermäßige Aufnahme von Fruktose fördert diesen Prozess [2]. Lipocalin 2 (LCN2) ist ein Transportprotein, das die Mobilisierung von Fettreserven reguliert und an der Kontrolle des Fetttröpfchen-assoziierten Proteins Perilipin 5 (PLIN5) beteiligt ist [3 – 5]. Lipopolysaccharide (LPS) führen in Hepatozyten sowohl zur Aktivierung des NLRP3 Inflammasoms sowie zur gesteigerten LCN2 Expression [6,7]. Ziele: Ziel der Studie ist es, das Netzwerk von NLRP3, LCN2 und PLIN5 in der Entstehung und Progression der NAFLD zu ergründen und dessen Beeinflussung durch Fruktose zu untersuchen. **Methodik:** HepG2 Zellen

und primäre Hepatozyten aus Wildtyp und LCN2-defizienten Mäusen wurden mit Fruktose oder LPS stimuliert. Die Expression von LCN2 und PLIN5 wurde mittels Westernblot und LCN2-Reporterassays untersucht. Die Aktivierbarkeit inflammatorischer Signalkaskaden wurde verglichen. **Ergebnisse/Schlussfolgerung:** Die Expression von LCN2 und PLIN5 steht in engem regulatorischen Bezug. In primären Hepatozyten führt eine Stimulation mit Fruktose oder LPS zu einer gesteigerten Induktion von LCN2 und PLIN5, die in HepG2-Zellen nicht beobachtet wird. HepG2 Zellen reagieren unempfindlicher auf entzündliche Reize und zeigen eine deutlich reduzierte Aktivierung von NLRP3. LCN2 und PLIN5 werden über inflammatorische Signalkaskaden reguliert und sind bei der Entstehung von NAFLD involviert. **Referenzen:** [1] Perry et al. Nature 2014;510:84 – 91. [2] Alwahsh et al. World J Gastroenterol. 2014;20: 1807 – 1821. [3] Zhao et al. J Biol Chem. 2014;289:5960 – 9. [4] Borkham-Kamphorst et al. Biochim Biophys Acta 2013;1832:660 – 673. [5] Asimakopoulou et al. Biochim Biophys Acta 2014;1842:1513 – 1524. [6] Boaru et al. J Inflamm. 2012;9(1):49. [7] Boaru et al. Biochem Biophys Res Commun. 2015;458:700 – 706.

1.29 LPS and bone morphogenetic protein (BMP)-9 regulate the hepatocytes acute phase response by affecting hepatic stellate cells

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Under conditions of liver damage (bacterial overgrowth in the gut but also damage induced by CCl4 injection or during regeneration after partial hepatectomy), LPS levels are elevated and lead to induction of the inflammatory response (= acute phase response; APR) in hepatocytes. BMP-9, a member of the TGF-β family of cytokines, is produced in the liver and we found that it has rather maturation inducing effects there, like enhanced polarization, inhibited proliferation and strengthened metabolic activity of hepatocytes. We further observed that the main BMP-9 producing cell type in the liver is the hepatic stellate cell, HSC (either quiescent or activated). Treatment of primary mouse HSC with LPS in vitro led to enhanced expression and secretion of pro-inflammatory cytokines, including IL6, IL-1β and TNFα but at the same time strongly reduced the expression of BMP-9. We then speculated that under conditions of damage where hepatocytes need to perform the acute phase response the above mentioned maturation effects of BMP-9 might be rather hindering for a proper wound-healing reaction. Therefore downregulation of BMP-9 (by LPS) might be needed to take the "break" off. And indeed we found that two days after partial hepatectomy in mice, a time-point where the hepatocytes are highly proliferative, BMP-9 levels are almost undetectable but return to normal (high) levels within a few more days. In conclusion our data point to a mechanism of LPS-mediated inflammation/wound-healing reaction in the liver that is centrally controlled by the BMP-9 levels produced by HSC.

1.30 Mast cells inhibit activation and profibrogenic activities of hepatic stellate cells

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Background: Mast cells (MC) have been implicated in the process of fibrosis in different organ systems [1]. Their biological effects are either based on an intimate relationship of MC and fibroblasts via soluble mediators [2, 3] or direct cellular interaction [4, 5]. **Methods and Results:** A direct co-culture of activated hepatic stellate cells (HSC) [6] and a murine MC line (L-138.8A) in the presence/absence of IL-3 and/or TGF-β1 was performed. Proteins of the supernatant, the adherent cells (HSC) and the suspension cells (MC) were analyzed by Western blot. In the presence of MC the expression of collagen IV (supernatant) and connective tissue growth factor (CTGF; supernatant and lysate) was markedly reduced. Furthermore, the expression of activation markers of HSC, e.g. α-SMA, was silenced. The corresponding TGF-β-receptors RII and Endoglin were downregulated which most likely is the basis for the reduced Smad signaling as evidenced by lower Smad2 phosphorylation (HSC). **Conclusions:** Direct co-culture of MC and HSC leads to a reduction in TGF-β1-signaling by causing reduced receptor expression and consecutive Smad activation. This leads to a block in HSC activation and matrix gene expression. **References:** [1] Overed-Sayer C, Rapley L, Mustelin T, Clarke DL. Are mast cells instrumental for fibrotic diseases? Front. Pharmacol. 2014;4:1 – 10. [2] Gaca MDA, Zhou X, Benyon C. Regulation of hepatic stellate cell proliferation and collagen synthesis by proteinase-activated receptors. J. Hepatol. 2002;36:362 – 369. [3] Dong X, Zhang C, Ma S, Wen H. Mast cell chymase in keloid induces profibrotic response via transforming growth factor-β1/Smad activation in keloid fibroblasts. Int. J. Clin. Exp. Pathol. 2014;7:3596 – 3607. [4] Gaca MDA, Pickering JA, Arthur MJP, Benyon RC. Human and rat hepatic stellate cells produce stem cell factor: a possible mechanism for mast cell recruitment in liver fibrosis. J. Hepatol. 1999;30:850 – 858. [5] Wygrecka M, Dahal BK, Kosanovic D, Petersen F, Taborski B, von Gerlach S, Didiasova M, Zakrzewicz D, Preissner KT, Schermuly RT, Markart P. Mast cells and fibroblasts work in concert to aggravate pulmonary fibrosis. Am. J. Pathol. 2013;182:2094 – 2108. [6] Meurer SK, Alsamman M, Sahin H, Wasmuth HE, Kisseleva T, Brenner DA, Trautwein C, Weiskirchen R, Scholten D. Overexpression of endoglin modulates TGF-β1-signalling pathways in a novel immortalized mouse hepatic stellate cell line. Plos One 2013;8:e56116.

1.31 Regulation and profibrogenic effects of TGF-β1 signaling in mast cells, a cell type contributing to the outcome of hepatic injury

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Background: In the setting of mastocytosis, mast cells have been shown to promote renal and lung fibrosis, whereas their effect in the liver is under debate [1 – 4]. Mast cell effects are based on the secretion of mitogenic and profibrotic, e.g. TGF-β1, cytokines – leading to activation and proliferation of fibroblasts [5] – and proteases – causing a direct or indirect modulation of extracellular matrix homeostasis [6]. Besides these paracrine effects, mast cells are able to process TGF-β1 signals on their own but the exact mechanisms and responses are less well characterized. **Material and Methods:** The impact of profibrogenic TGF-β signaling on mast cell biology was analyzed in primary bone marrow-derived murine mast cells (BMMC) and immortalized murine and human mast cell lines by qRT-PCR, western blot and immunocytochemistry. **Results:** BMMC and commonly used mast cell lines, e.g. human HMC1.1 and murine L-138.8A express the type I (ALK5) and type II TGF-β-receptors. Although ALK1 is only expressed in HMC1.1, Smad2/3 and Smad1/5 activation is seen in all mast cells. In line, the inhibitory/apoptotic effect of TGF-β1 on mast cell proliferation is blocked by the ALK5 inhibitor SB431542, showing that ALK5/Smad axis is critical for TGF-β1 signaling. On the molecular level TGF-β1 reduces the mRNA expression of cyclinE/A and c-myc in BMMC. In the presence of IL-3 the inhibitory effect of TGF-β1 on proliferation is reduced as is phosphorylation of Smads. Nevertheless, cross talk of TGF-β1 is also seen for the activation of STAT5, src and pp42/44. Finally, endoglin, i.e. a TGF-β1-co-receptor, is expressed only in BMMC and sparsely in L-138.8A but not in HMC1.1. Re-expression of endoglin in HMC1.1 modulates the signal transduction of IL-3 and TGF-β1. **Conclusions:** These results demonstrate that i) mast cells possess a versatile network to process TGF-β1 signals, ii) TGF-β1 impacts proliferation and differentiation of mast cells, iii) TGF-β1 affects several aspects of IL-3 signaling iv) these effects are modulated by endoglin, a modulatory TGF-β receptor expressed in primary BMMC. These data suggest that targeting TGF-β signaling may provide promising benefits in alleviating inflammatory or fibrotic effects of mast cells in liver injury. References cited: [1] Farrell DJ et al. Hepatology 1995;22:1175 – 81. [2] Armbrust T et al. J. Hepatol. 1997;26:1042 – 54. [3] Okazaki T et al. Lab. Invest. 1998;78:1431 – 8. [4] Sugihara A et al. J. Hepatol. 1999;30:859 – 67. [5] Evans RA et al. Exp. Cell Res. 2003;282:90 – 100. [6] Dong X et al. J. Clin. Exp. Pathol. 2014;7:3596 – 607.

1.32 Overwhelming high quantities of the matricellular protein CCN1/CYR61 induce ER stress-related cellular apoptosis in hepatic stellate cells

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Background: CCN1/CYR61 is a matricellular protein belonging to CCN protein family that contains six secreted proteins associated with extracellular matrix signaling [1]. It acts as an enhancer of cutaneous wound healing process by preventing hypertrophic scar formation through induction of myofibroblast (MFB) senescence [2]. In liver fibrosis, senescent cells are primarily derived from activated hepatic stellate cells (HSC) that initially proliferate in response to liver damage and transdifferentiate into MFB. Activated HSC and transdifferentiated MFB are one major source of CCN1 [3]. **Methods:** We tested if CCN1 act as a senescence inducer to attenuate liver fibrogenesis by means of adenoviral CCN1 gene transfer in primary HSC, MFB, CFSC-2G, and LX-2. **Results:** The overwhelming concentrations of CCN1 protein resulted in an overload of the endoplasmic reticulum (ER) and compensatory unfolded protein response (UPR) in all these cells. The UPR resulted in upregulation of ER chaperones (BIP/Grp78, Grp94) and leads to an activation of IRE1α as evidenced by spliced XBP1 mRNA with IRE1α-induced JNK phosphorylation. The UPR arm PERK and eIF2a was phosphorylated, and CHOP upregulated. Ad5-CMV-CCN1 induced HSC apoptosis by proteolytic cleavage of caspase-12, caspase-9, and caspase-3 resulting in positive TUNEL stain. Remarkably, CCN1 effectively blocked collagen type I expression at both mRNA and protein levels. **Conclusions:** High concentrations of the matricellular protein CCN1 induce HSC apoptosis through ER stress and UPR. Therapeutic CCN1 gene transfer might be useful to mitigate liver fibrosis when cell-specific targeted to HSC and MFB. References cited: [1] Weiskirchen R. Front Biosci 2011;16:1939 – 61. [2] Jun JI & Lau LF. Nat Cell Biol 2010;12:676 – 85. [3] Borkham-Kamphorst E et al. Biochim Biophys Acta 2014;1843:902 – 14.

1.33 Platelet integrin-dependent fibrillogenesis of fibronectin: Impact of shear stress

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Introduction: Soluble plasma fibronectin (Fn) with its inactive compact structure requires unfolding to assemble into active fibrils. Fibril formation of Fn is a cell-mediated process. Less is known about the contribution of biomechanical forces on the fibrillogenesis of Fn. The aim of this study was to investigate conformational changes of Fn, as induced by platelet integrins and/or shear rates simulating venous or arterial flow conditions. **Methods:** Human plasma Fn, in the presence or absence of washed platelets, was added to plates pre-coated with Fn. Subsequently, the solutions were exposed to shear using a cone-plate rheometer (Haake Rheostress 1). For microscopic analysis (LSM 510, Carl Zeiss), Alexa flour 488-conjugated Fn was used. In parallel experiments, a N-terminal 70kDa fragment of Fn was incubated with soluble Fn before exposure to shear. To examine the role of distinct platelet integrins on fibril formation of Fn, washed platelets were incubated with monoclonal antibodies LM609, P1D6, 10E5, or abciximab (10 µg/ml, each) to block αvβ3, α5β1, αIIbβ3, or both αIIbβ3 and αvβ3, prior to the addition of Fn and subsequent exposure to shear. In all experiments, flow conditions were simulated by shear rates, stepwise increasing from 50 1/s to 5000 1/s within 5 min and subsequently decreasing from 5000 1/s to 50 1/s within 5 min. To study the structure of Fn fibrils, solutions were examined by laser scanning microscopy after exposure to shear. To quantify the amount of fibril formation, deoxycholate solubility assays and densitometric analysis of Western blots were performed. Control experiments were conducted under static conditions. **Results:** Microscopic analyses showed that exposing Fn solutions to shear resulted in fibril formation. Fn fibril diameter varied from 0.5 to 5 µm. Observed fibrils were linked with each other and varied in length (from 50 to 300 µm). Treatment of Fn with the N-terminal 70 kDa fragment of Fn, which is known to inhibit Fn matrix assembly, blocked fibril formation of Fn. Western blotting and densitometric analyses revealed that addition of washed platelets to Fn solution resulted in increases of 20-fold in fibril formation of Fn (calculated as the ratio of insoluble to soluble Fn), generated by shear ($p < 0.05$, $n = 3$). In contrast, 10E5 or abxicimab blocking αIIbβ3, or both αIIbβ3 and αvβ3 caused a reduction by 82% or 74% in fibril formation of Fn ($p < 0.05$, $n = 3$ each), in comparison to samples without antibodies. Blocking α5β1 or αvβ3 by P1D6 or LM609 only caused a reduction by 17% or 56% ($p > 0.05$, $n = 3$ each). Under static conditions, no fibril formation was detected. **Conclusions:** Our results indicate that fibrillogenesis of Fn is modulated by shear conditions. Furthermore, formation of fibrils is induced by platelet integrins. Hereby, αIIbβ3 plays a predominant role, while α5β1 has a minor part among the three examined platelet integrins with regards to Fn fibril formation.

1.34 Promotion of macrophage activation and inflammation in chronic liver disease by the histidine-rich glycoprotein

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Danger signals and cytokines released by immune cells influence the functional differentiation of macrophages in chronic inflammation. Recently, the liver-derived plasma protein histidine-rich glycoprotein (HRG) was demonstrated to mediate the transition of alternatively activated (M2) to pro-inflammatory and anti-tumoral (M1) macrophages. We hypothesized that liver-derived HRG is a critical mediator of hepatic macrophage functionality and therefore studied its role in chronic liver disease using mouse models of hepatic fibrosis in Hrg-/- and wildtype (WT) mice. In steady state, hepatic macrophages were reduced polarized towards the M2 subtype in Hrg-/- mice. Upon chronic liver damage induced by CCl4 or methionine-choline-deficient (MCD) diet, liver injury and fibrosis were attenuated in Hrg-/- compared to WT mice. Macrophage populations were reduced and skewed towards M2 polarization in injured livers of Hrg-/- mice. Moreover, HRG-deficient mice showed significantly enhanced hepatic vascularization by micro-computed tomography (µCT) and histology, as a result of M2 activation of hepatic macrophages. Purified HRG protein induced, but HRG-deficient serum prevented, M1 macrophage differentiation in vitro. Accordingly, Hrg/- mice transplanted with Hrg+/+ bone marrow, but not Hrg-/–transplanted Hrg +/+ mice, remained protected from experimental steatohepatitis. Based on these data, we studied HRG expression and macrophage polarization in human liver biopsies by immunohistochemistry. Consistent with our findings from animal models, patients with chronic hepatitis C significantly upregulated hepatocytic HRG expression, which was associated with M1 polarization of adjacent macrophages. In conclusion, liver-derived HRG appears to be a promising therapeutic target for chronic inflammatory liver diseases.

1.35 Multiple quantitative trait loci modeling (MQM) of hepatic fibrosis in a murine intercross

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Most common diseases are attributed to multiple genetic variants. The feasibility of identifying inherited risk factors is often restricted to the identification of alleles with high or intermediate effect sizes. In our previous studies we identified single susceptibility loci associated with hepatic fibrosis (Hfib1-Hfib4). New advances in analysis tools allow us to model loci interactions for liver fibrosis. Overall we analysed 322 progeny from an F2 intercross of the fibrosis-susceptible inbred mouse strain BALB/cJ and the resistant strain FVB/NJ. The mice were challenged with carbon tetrachloride (CCl4) for six weeks to induce liver fibrosis. We quantified fibrosis progression by determining histological fibrosis stages and hepatic collagen (hydroxproline) contents. Phenotypic data were correlated to genome-wide markers to identify quantitative trait loci (QTL). Thirteen susceptibility loci were localized by single and composite interval mapping, and were included in the subsequent multiple QTL model (MQM) testing. Models provided evidence for susceptibility loci with strongest association to hydroxyproline (chromosomes 1, 2, 8 and 13) or fibrosis stages (chromosomes 1, 2, 12 and 14). These loci contain the fibrosis modifier genes Hc, Fasl, and Foxa2. The MQM were displayed as a fibrosis network. Interestingly, we identified only one overlapping

hepatic fibrosis locus on chromosome 1 for both phenotypes. Including MQM to association studies adds valuable information on gene-gene interactions and may eventually be accomplished in human cohorts. This study presents an initial step towards a refined understanding of profibrogenic gene networks.

1.36 Shear-induced fibrillar-like supramolecule of plasma fibronectin: A new form of fibronectin with enhanced activity in platelet adhesion and aggregation

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Background: We reported on the formation of fibronectin fibrils (FN fibrils) induced by shear rates. Aims: Here, we characterized the function of shear-induced FN fibrils in platelet adhesion and aggregation. **Methods:** For adhesion experiments, CMFDA labeled platelets were placed onto immobilized FN or FN fibrils (25 µg/ml) for 30 min at 37 °C. In parallel experiments, platelets resuspended in FN-depleted plasma were placed onto immobilized FN, collagen, or fibrinogen (10 µg/ml) in the presence of soluble FN (300 µg/ml) or FN fibrils (10 µg/ml). For aggregation experiments, FN (5, 10, 300 µg/ml) or FN fibrils (5, 10 µg/ml) was added to PRP or platelets resuspended in FN-depleted plasma. Aggregation was induced by 400 nM PMA, or 10 µg/ml collagen. **Results:** The adhesion rates of washed platelets were higher onto surfaces coated with FN fibrils than with plasma FN. In parallel experiments using platelets resuspended in FN-depleted plasma, addition of plasma FN (300 µg/ml) increased platelet adhesion onto immobilized FN (from 0.14 ± 0.01 to 0.18 ± 0.02, p = 0.04), collagen (from 0.14 ± 0.005 to 0.2 ± 0.01, p = 0.0007), and fibrinogen (from 0.16 ± 0.03 to 0.22 ± 0.01). Addition of FN fibrils (10 µg/ml) had a similar supportive effect. Conversely, FN showed an inhibitory effect in platelet aggregation. Activation by PMA induced aggregation of PRP by 81%. In the presence of plasma FN at 5, 10, 300 µg/ml, platelet aggregation was reduced to 50%, 41%, and 29.5%, respectively. A stronger inhibition on platelet aggregation was seen with FN fibrils. PRP aggregated by 35.4% and 17% in the presence of 5 and 10 µg/ml FN fibrils, respectively. The same phenomenon was observed in aggregation assays using platelets resuspended in FN-depleted plasma and collagen as activating agonist. **Conclusion:** Our study suggested that shear-induced FN fibrils have stronger activity in supporting platelet adhesion and inhibiting platelet aggregation than normal plasma FN. This finding emphasizes the importance of FN assembly on its activity in platelet function.

1.37 Shear-related fibrillogenesis of fibronectin

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Background: Biomechanical forces can induce transformation of fibronectin from its compact structure to an extended fibrillar state. Adsorption of plasma proteins onto metallic surfaces may also influence their conformation. **Methods:** We used a cone-plate rheometer to investigate the effect of shear and stainless steel on conformational changes of fibronectin. In control experiments, cones grafted once or twice with polyethylene glycol were used. Plasma fibronectin was added at concentrations of 50 or 100 µg/ml to BSA- or fibronectin-coated plates and subsequently exposed to dynamic shear rates, stepwise increasing from 50 1/s to 5000 1/s within 5 min and subsequently decreasing from 5000 1/s to 50 1/s within 5 min. Viscosity (mPa s) of fibronectin solutions was recorded over 10 min. **Results:** Upon exposure to shear, the viscosity in the sample increased, suggesting conformational changes in fibronectin. Western blotting and densitometric analyses demonstrated that conformational changes of plasma fibronectin depended both on shear and protein concentration. However, there was no significant difference in fibril formation between BSA- or fibronectin-coated plates, suggesting that physical properties of stainless steel and biomechanical forces such as shear can affect the molecular structure of fibronectin. **Conclusion:** Our model may provide useful information of surface- and flow-induced alterations of adhesive proteins.

1.38 The bile acid-phospholipid conjugate Ursodeoxycholyl Lysophosphatidylethanolamide (UDCA-LPE) disturbs pro-fibrogenic Integrin and TGFβ signaling

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Background: Integrin receptors, which are involved in cell-cell and cell-matrix interaction emerge as crucial mediators of TGFβ1 activation in liver fibrosis. Ursodeoxycholyl Lysophosphatidylethanolamide (UDCA-LPE) is a synthetic bile acid-phospholipid conjugate with hepatoprotective and anti-fibrogenic functions in vitro and in vivo. In this study we aim to elucidate signaling pathways, which mediate anti-fibrogenic action of UDCA-LPE. **Results:** In order to promote pro-fibrogenic signaling upon extracellular matrix binding integrins recruit focal adhesion kinase (FAK) and SRC kinase, which are phosphorylated in response to integrin engagement. Incubation of CL48 liver cells and Human Hepatic Stellate cell (HHStec) with UDCA-LPE altered the localization of integrin α2, α3, α5, αv, β1, β3, β5 and β6 as observed by immunofluorescence. After UDCA-LPE treatment integrins were transported to the ER and the nuclear envelop, which led to a loss of co-localization of integrins with SRC resulting in dephosphorylation of FAK (Tyr 925 and Tyr 576/577) and SRC (Tyr416). This translocalization of integrins was not induced by UDCA and/or LPE treatment, but was exclusively achieved by UDCA-LPE. To further dissect whether UDCA-LPE would mediate a shift of integrins to certain membrane microdomains such as lipid raft and non-raft regions lipid fractioning was performed. Integrin α2, α3, α5, αv, β1, β4, but not SRC, was significantly reduced in transitional fractions and increased in lipid raft fractions. Taken together, UDCA-LPE disturbed integrin-FAK signaling after short activation by modifying the localization of integrins. Incubation with the integrin blocking peptide RGD inhibited UDCA-LPE induced translocalization of integrins, suggesting that integrins may bind UDCA-LPE with the RGD recognition motif. UDCA-LPE further led to a shift of TGFβ1 receptor I and II to the ER and the nuclear envelope resulting in dephosphorylation of Smad2/3. According to lipid fractionation TGFβ1 receptor I and II were also reduced in transitional fractions and increased in lipid raft fractions after UDCA-LPE treatment. Integrin blocking peptide RGD inhibited UDCA-LPE induced dephosphorylation of Smad2/3, suggesting that inhibition of TGFβ signaling was integrin dependent. Analysis by HPLC-MS revealed that UDCA-LPE moved from UDCA localization to LPE localization, corresponding with integrins and TGFβ1 receptors. **Conclusions:** UDCA-LPE mediated translocation of integrins and TGFβ1 receptors into lipid rafts leads to a loss of colocalization with their down-stream signaling proteins SRC and Smad2/3. By inhibiting crucial pro-fibrogenic signaling pathways UDCA-LPE emerges as a promising experimental drug-candidate for the treatment of liver fibrosis.

1.39 The TGR5 protein amount is reduced in patients with primary sclerosing cholangitis (PSC)

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Introduction: The localization of TGR5 in cholangiocytes, the choleretic, anti-inflammatory and anti-apoptotic functions of the receptor suggest that TGR5 is important for the pathogenesis of biliary diseases. Additionally, for the biliary tract disease PSC an association with the frequent TGR5 exon 1 SNP rs11554825 is described, which appears with a lower TGR5 mRNA expression (measured in lymphoblastic cell line) (Hov et al., 2010). Currently no data about TGR5 localization and expression in PSC patients are known. The milieu in PSC livers is insufficiently characterized, but elevated bile salt and cytokine levels are observed in chronic inflammatory bile duct disease. **Methods:** TGR5 protein localization was analyzed using immunofluorescence staining in livers from PSC patients

and controls as well as from MDR2-/- and WT mice. TGR5 protein amount in CK7 or CK19 positive cholangiocytes was determined by analysis of the mean fluorescence intensity per unit area (Axios Visio 4.8 software). TGR5 mRNA expression was quantified by qPCR in relation to an endogenous control (HPRT1), macrophage (CD 14, CD 163) or a cholangiocyte marker (CK19). To elucidate the TGR5 downregulation in livers macrophages isolated from blood mononuclear cells (PBMC) were used. Stimulation experiments with the most potent TGR5 bile acid ligand taurolithocholate (TLC), and the therapeutically used tauroursodeoxycholate (TUDC) and the cytokines TNFα and IL1β with subsequent TGR5 mRNA expression analysis were performed. **Results:** The immunofluorescence analysis showed a significant reduction in TGR5 protein levels in the bile ducts of the PSC livers and in the bile ducts of MDR2-/- knockout animals compared to controls of human livers and mouse livers. In both studies, there was no change in the protein level of CK7 per bile duct. In contrast analysis of whole liver mRNA did not reveal a significant reduction in TGR5 mRNA in PSC or MDR2-/- livers as compared to the respective controls. However, mRNA for CD 14 and CK19 also increased in livers from PSC patients or MDR2-/- mice. In chronic inflammatory biliary tract diseases bile salt and cytokine levels are increased. While the bile salts TLC and TUDC had no effects on TGR5 mRNA in isolated human macrophages, the inflammatory cytokines TNFα and IL1β significantly supressed TGR5 mRNA expression in these cells. **Discussion:** Using immunofluorescence staining and quantification we can demonstrate a downregulation of TGR5 protein amounts in biliary epithelial cells of livers from PSC patients and MDR2-/- mice. Whether this decrease in TGR5 protein levels is caused by reduced TGR5 mRNA expression is unclear. However, stimulation of TGR5 expressing human macrophages with TNFα and IL1β significantly suppressed TGR5 mRNA levels, while various bile acids had no effect on the receptors mRNA expression. Since TGR5 exerts protective effects in liver and cholangiocytes the downregulation of the receptor in PSC livers may render cholangiocytes more susceptible to bile acid toxicity and may also explain why feeding of a TGR5 agonist (INT-767) failed to alleviate liver damage in MDR2-/- mice (Baghdasaryan et al., 2011).

1.40 WISP1 modulates immune cell infiltration upon drug-induced liver injury (DILI)

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Multiple potentially harmful stimuli challenge the liver, the chief metabolic and detoxifying organ of the body. Administration of hepatotoxic chemicals (e.g., CCl4, APAP) can induce the loss of liver mass which may lead to fatal conditions such as acute liver failure, or to chronic pathological conditions such as fibrosis, cirrhosis and hepatocellular carcinoma. Drug metabolites cause direct cell stress, trigger specific inflammatory response which removes tissue debris, followed by the regenerative response. However, an excessive inflammatory response can lead to a dramatic aggravation of the existing injury. To design interventions, which selectively target the potential detrimental effects of immune cell infiltration into damaged tissue, a detailed understanding of the pathophysiology is critical. To unravel molecular mechanisms involved in the process of damage and regeneration, we performed gene array analyses of mouse liver tissue after single dose of CCl4 administration (1.6 g/kg body weight, i.p.) using the Affymetrix A420 2.0 gene chips. We observed an induction of a matricellular protein WISP1 during acute liver damage in mouse liver between 2 h and day 1, which returned to normal levels by day 3. Genetic deletion of WISP1 caused increased liver sensitivity to damage upon CCl4 intoxication and strongly influenced the expression of inflammatory mediators such as release of cytokines and immune cell infiltration. A clear increase in macrophage and neutrophil infiltration in WISP1 KO compared to control mice was observed by immunostainings as well as a multi-color flow cytometry screen for macrophages, monocytes, neutrophils, dendritic cells, NK cells, NKT cells, T helper and cytotoxic T cells, γδ T cells and eosinophils. In addition to the CCl4-model of acute liver damage, we currently investigate whether WISP1 influences the inflammatory response after hepatotoxic but not lethal dose of APAP injection (300 mg/kg body weight, i.p.), where WISP1 expression is induced at day 2 after APAP-induced damage. The time resolved analysis (12 h, 1, 2, 3, 6, and 12 days after administration of APAP) will show the kinetics of the individual infiltrating immune cell types. Together, this will deliver comprehensive and complementary information on the spatio-termporal impact of WISP1 on liver-immune cell interactions in the context of liver damage. In conclusion, we have strong evidence that WISP1 plays a yet unrecognized role in liver injury. Our data suggests that WISP1 is modulating the onset of inflammation and the recruitment of leukocytes (i.e. neutrophils and macrophages) upon drug-induced liver injury.

1.41 Therapeutische Effekte der Modulation des Endocannabinoid-Rezeptor Signalwegs

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Einleitung: Das körpereigene Endocannabinoid-System ist an der Kontrolle chronischer Entzündungsprozesse in der Leber beteiligt. In Tierstudien zu toxischen Lebererkrankungen, konnte durch Antagonisierung des Endocannabinoid-Rezeptors-1 (CB1) bei chronischer Schädigung ein deutlicher Rückgang der Fibrose bewirkt werden. Mit der vorliegenden Studie sollte die potentiell profibrotische Wirkung des CB1-Agonisten ACEA -und die potentiell antifibrotische Wirkung des CB1- Antagonisten Rimonabant am Abcb4-/- Mausmodells untersucht werden. **Methoden:** Männliche Abcb4-/–Mäuse wurden nach Absetzen vom Muttertier mit ACEA bzw. Rimonabant bis zu einem Alter von 16 bzw. 52 Wochen behandelt. Leber, Serum und abdominales Fett wurden isoliert und mittels Serumanalytik, Micro-Array, RT-PCR, Western Blot und Immunhistochemie untersucht. Als Kontrolle dienten unbehandelte Abcb4-/- Mäuse und Balb/C Wildtypen. **Ergebnisse:** Im mRNA Array wurde bei den behandelten Mäusen eine Normalisierung von ca. 60% der durch den Abcb4-Knockout regulierten Gene beobachtet. Sowohl die Behandlung mit ACEA, als auch die mit Rimonabant verbesserte die Leberintegrität (Reduktion von GPT) und die Fibrose (Abnahme des Hydroxyprolingehalts). Zusätzlich wurden auf metabolischer Ebene weitgehend gleichgerichtete Effekte durch die Agonisierung und Antagonisierung des CB1-Rezeptors beobachtet (PPARa, PPARg, PEPCK, FASN, Triglyzeridmessungen in Serum und Leber). **Schlussfolgerung:** Die vorliegende Studie zeigt, dass sowohl eine Stimulation, als auch eine Antagonisierung des CB1 Rezeptors gleichgerichtete hepatoprotektive Effekte auf die fibrotische Leber im Abcb4-/- Mausmodell haben. Die zugrundeliegenden Mechanismen dieser überraschenden Ergebnisse werden in weiteren Untersuchungen analysiert.

1.42 MicroRNA-221 inhibition in hepatocytes ameliorates liver fibrosis

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Excessive extracellular matrix accumulation due to chronic liver injury leads to fibrosis, cirrhosis and may then eventually, progress to hepatocellular carcinoma. Small non-coding microRNAs (miRNAs) play important roles in the regulation of multiple liver functions and diseases. Among various miRNAs, miR-221 has recently been reported to be upregulated in liver fibrosis, and its expression correlates with severity of liver fibrosis in HCV patients. We show that downregulation of miR-221 in hepatocytes can ameliorate liver fibrosis by decreasing the activation of HSCs. We inhibited miR-221 expression by an adeno-associated virus encoding a tough decoy against miR-221 (AAV TuD). Reduced levels of serum transaminases and hepatic fibrotic markers were found in mice injected with AAV TuD. Therefore, our findings indicate that suppression of miR-221 in hepatocytes ameliorates liver fibrosis. Our study provides a novel therapeutic approach for the treatment of liver fibrosis via miRNA modulation.

2. Clinical Hepatology

2.1 Fibrosis regression in autoimmune hepatitis

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Background: A primary objective of immunosuppressive therapy in autoimmune hepatitis (AIH) is to prevent the progression of liver fibrosis. In patients with viral hepatitis it is now accepted that after control of the virus fibrosis may regress, even in patients with prior liver cirrhosis. Aims: to assess changes of liver fibrosis in AIH patients over time by using transient elastography (TE) and liver biopsy. In addition, we compared fibrosis development in AIH with the development in primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC). **Methods:** In a pilot study we validated the diagnostic performance of TE in AIH in relation to liver histology. A total of 130 patients with AIH, 57 patients with primary biliary cirrhosis (PBC) and 58 patients with primary sclerosing cholangitis (PSC) underwent two TE measurements separated by a minimum interval of 1 year. The first TE measurement included in AIH patients was obtained after a minimum of 6 months of treatment, thereby minimizing the influence of liver inflammation on TE values. In addition, we analysed liver biopsies from 64 AIH patients who had undergone at least two consecutive liver biopsies guided by minilaparoscopy. **Results:** Liver stiffness strongly correlated with the histologic fibrosis score according to Desmet and Scheuer classification (Spearman's $\rho = 0.611$, $p < 0.001$) and showed high accuracy in predicting cirrhosis with an optimal cutoff of 16.05kPa (AUROC 0.92, Sensitivity: 0.85, Specificity: 1.00). Changes in liver stiffness were -1.17 ± .32 kPa/year in AIH, +0.025 ± 1.4 kPa/year in PBC and +0.61 ± 3.6 kPa/year in PSC. Average time between TE measurements was 2.5years (range:1 – 6ys), 3.9 years (range:1 – 7) and 2.3 years (range:1 – 6), respectively. None of the patients with AIH and consecutive liver stiffness measurements developed signs of hepatic decompensation (ascites/varices/hepatic encephalopathy/splenomegaly). Decrease of liver-stiffness over time was a prognostic factor for the achievement of biochemical remission at last follow-up (OR 10.2, $p = 0.003$). Age at diagnosis < 18 was linked to progression of liver stiffness ($p = 0.037$, OR = 4.1). The majority of follow-up biopsies were obtained because of incomplete remission, thereby introducing selection bias. Even in this study cohort, 50% of patients showed stable disease and 28% regression of fibrosis. In 7/12 58%) cases of histologically proven cirrhosis a reversal of cirrhosis could be observed. **Conclusions:** (i) Transient Elastography is able to differentiate severe from none-severe liver fibrosis and is a valuable tool in disease monitoring in patients with AIH (ii) In contrast to PSC and PBC, a large proportion of patients with AIH demonstrates regression of liver stiffness and histologically proven fibrosis stage (iii) Regression of fibrosis should be regarded as an achievable goal in the treatment of AIH. (iv) Diagnosis < 18years is associated with progression of liver fibrosis.

2.2 Determining the molecular consequences of clinically relevant glutamine synthetase mutations

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Glutamine synthetase (GS) catalyzes the ligation of ammonia and glutamate to glutamine under the use of ATP and is, thus, essential for nitrogen metabolism [1,2]. Loss of hepatic GS activity has been linked to serious clinical conditions [3]. In particular, two mutations of human GS (R324C and R341C) were connected to congenital glutamine deficiency with severe brain malformations resulting in neonatal death [4]. In a single case known to date, to the best of our knowledge, another GS mutation (R324S) was identified in a neurologically compromised patient [5]. However, the underlying molecular mechanisms of GS deactivation by these mutations have not been understood yet. By means of molecular dynamics simulations, free energy calculations, and rigidity analyses we found that all three mutations influence ATP binding, the first step of GS glutamine formation cycle. In the case of the R324S and R324C mutants, we found a loss of direct salt-bridge interactions with the substrate ATP. This hampers ATP binding and causes a deterioration of GS catalytic activity. Remarkably, in the case of the R324S mutant, we observed water-mediated interactions with ATP that reduce this effect and may explain the suggested higher GS residual activity [6]. The R341C mutation destabilizes residue R340 that is important for ATP binding. Furthermore, we predicted the R341C mutant to result in a significant destabilization of helix H8, which should hamper glutamate binding. To further corroborate this hypothesis, we introduced an additional GS variant by alanine mutagenesis of amino acids interacting with R341, mimicking the loss of interactions in the R341C mutant. After GS overexpression in HEK293 cells, dot-blot analyses revealed that the structural stability of H8 was impaired in the case of the newly introduced GS mutant. This results in a loss of masking of the epitope in the glutamate binding pocket for a monoclonal anti-GS antibody by L-methionine-S-sulfoximine; in contrast, cells transfected with wild type GS did show the masking. Our analyses show complex molecular effects underlying GS deactivation in three clinically relevant mutants. Furthermore, since there is currently no adequate therapy available [6] to treat a glutamine deficiency caused by the R324S mutant, our findings could stimulate the development of ATP binding-enhancing molecules by which the R324S mutant can be "repaired". We are grateful to the "Zentrum für Informations und Medientechnologie" (ZIM) at the Heinrich Heine University for computational support. This work was supported by the Deutsche Forschungsgemeinschaft through the Collaborative Research Center SFB 974 ("Communication and Systems Relevance during Liver Damage and Regeneration", Düsseldorf). **References:** [1] Häussinger, D.: Eur. J. Biochem. 1983, 133(2): 269 – 275 [2] Häussinger, D.: Biochem. J. 1990, 267(2): 281 – 290 [3] Qvartskhava, N. et al.: P. Natl. Acad. Sci. USA 2015, 112 (17): 5521 – 5526 [4] Häberle, J. et al.: New. Engl. J. Med. 2005, 353(18): 1926 – 1933 [5] Häberle, J. et al.: Mol. Genet. Metab. 2011, 103(1): 89 – 91 [6] Häberle, J. et al.: Orphanet J. Rare. Dis. 2012, 7(48): 1 – 10

2.3 Urine cell-derived hepatocyte-like cells as potential therapeutic cell transplants for different liver diseases

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Hepatocytes derived from human somatic cells would be useful in regenerative medicine, drug development and cell-based disease models. Several types of somatic cells have been reprogrammed to induced pluripotent cells (iPSCs) and then differentiated to hepatocyte-like cells (iHeps). However, the method for generating such cells from renal epithelial cells shed in human urine has not been described systematically. Thus, we tested whether these urine cell-derived iPS cells will show the ability to differentiate into iHeps and may have the potential to engraft in mouse liver. 250 – 500 ml of fresh human urine was collected from 3 different donors. After several washing steps, the cell pellet was cultivated in one well of a 12 well plate. The isolated epithelial cells were reprogrammed into iPS cells by using transgene-free methods, all delivering the pluripotency factors Oct3/4, Sox2, Klf4 and c-Myc. After characterization of stable iPS cell lines, we started a three-step protocol for hepatic differentiation. To evaluate hepatic status during differentiation process we analyzed 94 genes using qPCR, as well as flow cytometry analysis, immunocytochemistry and hepatocyte-specific functional assays. One million iHeps were transplanted via spleen into PiZ/Scid mice, a model for alpha 1-antitrypsin deficiency. 5 days after urine-cell cultivation, 4 – 6 cell colonies were observed which outgrew to a stable cell population within the next 2 weeks. Reprogramming of urine cells produced iPS cell lines that indicated typical stem cell features. The first step of differentiation induced a substantial morphological change, while 90% of the cells expressed the definitive endoderm marker Sox17, shown by qPCR and immunocytochemistry. At the final stage, flow cytometry analysis revealed 86% of Albumin and 29% of ASGPR positive cells. Genes which are responsible for cholesterol homeostasis, bile transport and

detoxification processes are upregulated, e.g. farnesoid X receptor (FXR), bile sort export pump (BSEP; ABCB11) and constitutive androstane receptor (CAR), shown on mRNA level. The iPS-derived iHeps showed glycogen storage, urea and albumin production. Engraftment of iHeps in livers of PiZ/Scid was proofed by immunohistochemistry. The results indicate that urine cell-derived iPS cells can be efficiently induced to differentiate into iHeps. We found that our methods allowed the expression of liver specific functions, which will be beneficial as potential therapeutic cell transplants for various liver diseases.

2.4 A pro-inflammatory role of type 2 innate lymphoid cells in murine immune-mediated hepatitis

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Type 2 innate lymphoid cells (ILC2) mediate inflammatory immune responses in the context of diseases triggered by the alarmin IL-33. We observed high levels of IL-33 in serum of patients with acute hepatitis B infection, suggesting a role for this cytokine in disease pathogenesis. However, the immunoregulatory functions of ILC2 s and IL-33 in immune-mediated hepatitis are less clear. Using the murine model of Concanavalin (Con)A-induced immune-mediated hepatitis, we showed that elevated hepatic IL-33 expression was associated with severe liver inflammation. Interestingly, ILC2 s expressing the IL-33 receptor ST2 selectively expanded in the inflamed liver and expressed the type 2 cytokines IL-13 and IL-5. ILC2 depletion correlated with less severe hepatitis whereas adoptive transfer of IL-33-elicited ILC2 s aggravated liver damage. We further showed that short-term IL-33 treatment before Con A challenge caused more severe liver inflammation. In contrast, long-term IL-33 treatment potently suppressed development of immune-mediated hepatitis and was accompanied by the expansion of both ILC2 s and ST2+ Foxp3+ regulatory T cells. This subset of regulatory T cells also accumulated in the inflamed livers of Con A-treated mice. **Conclusion:** ILC2 s are poised to respond to the release of IL-33 upon liver tissue damage through expression of type 2 cytokines thereby contributing to the pathogenesis of immune-mediated hepatitis. IL-33 exerts a dual function in immune-mediated hepatitis, acting on ILC2 s in order to promote disease pathology, while concurrently regulating inflammatory responses by eliciting the generation of ST2+ regulatory T cells.

2.5 Ascites total protein may be modulated by the use of diuretics

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Background and Aims: Low total protein in the ascites has been described as a risk factor for spontaneous bacterial peritonitis (SBP). However, recent studies showed that also patients with high ascites protein are susceptible for SBP. Diuretics have been suggested to increase the amount of total protein in the ascites. We investigated if drugs that are commonly used in patients with liver cirrhosis, stage of disease and presence of complications may influence the amount of total ascites protein. **Methods:** Clinical and laboratory data from patients with liver cirrhosis who received paracentesis between March 2012 and October 2014 in our department were analysed for factors associated with total protein in the ascites. **Results:** In total, 273 patients were analysed. 174 (64%) were male, most patients (168, 62%) had cirrhosis due to alcohol abuse. According to Child-Pugh classification, 1%, 52% and 47% of patients had cirrhosis stage A, B and C, respectively. Median total protein in the ascites was 12 g/L (range 2 – 48 g/L). 36 patients had SBP at inclusion and were excluded from further analysis. Patients with Child-Pugh-Stage B (n = 130) had higher levels of total protein than patients with stage C (median 15 versus 9 g/L, $p < 0.001$). As reported previously, ascites total protein did not differ between patients with SBP either in the past or during follow-up (n = 58) compared to patients without occurrence of SBP (11 versus 12 g/L; $p = 1.0$). Ascites protein levels were also comparable for patients with (n = 39) or without hepatocellular carcinoma (10 versus 13 g/L; $p = 0.17$), portal vein thrombosis (n = 36; 14 versus 12 g/L; $p = 0.8$) and recurrent hepatic encephalopathy (n = 44; 11.6 versus 12 g/L; $p = 0.3$). However, active variceal bleeding (n = 18) was associated with lower ascites protein levels (9.5 versus 12 g/L; $p = 0.005$). Comparing patients with and without the respective drug, total protein in the ascites was comparable for patients taking proton pump inhibitors (n = 191) or not (11.6 versus 12 g/L; $p = 0.7$), beta-blockers (n = 112; 11 versus 12 g/L; $p = 0.7$) or rifaximin (n = 25; 11.6 versus 12 g/L; $p = 0.7$). However, in patients taking diuretics (n = 161), increased levels of total ascites protein were found (14 versus 9 g/L; $p < 0.001$). Creatinine in the serum did not correlate with total ascites protein ($r = 0.06$; $p = 0.4$). When entering Child-Pugh-Stage, active variceal bleeding and use of diuretics into a multiple linear forward regression analysis, all factors were significantly associated with the amount of total ascites protein. When patients were stratified for a threshold of 10 g/L total ascites protein, Child-Pugh stage B, use of diuretics and absence of active variceal bleeding were associated with ascites total protein levels above 10 g/L in uni- and multivariate analysis. **Conclusion:** The impact of diuretics on the levels of ascites total protein may hamper its predictive capacity for occurrence of SBP.

2.6 Beneficial effects of IL-1 cytokine inactivation and the role of the serine/threonine kinase MK-2 in hepatic steatosis in a murine obesity model

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Background and Aims: Although non-alcoholic fatty liver disease (NAFLD) is among the most common causes of chronic liver disease worldwide, its pathogenesis is yet poorly understood. Recent studies show that the pro-inflammatory cytokines IL-1αand IL-1β play a crucial role in disease development. Their recognition by cells does not only result in transcriptional regulation of many IL-1 target genes, but also negatively affects cell surface expression of the IL-6 signal transducer gp130 in an MK-2 (mitogen-activated protein kinase-activated protein kinase 2)-dependent mechanism. Therefore the cells might become less susceptible to IL-6 signalling, which is suspected to have a beneficial effect in steatosis. We aim to investigate the role of IL-1 in early steatosis in IL-1-immunized and MK-2 deficient mice. **Methods:** C57BL/6J wild-type and MK-2 deficient mice were fed a Surwit High Fat Diet (HFD) for 8 weeks. For inactivation of IL-1, mice were subcutaneously vaccinated against IL-1α/β using virus-like particles presenting antigens of IL-1 α or β (Cytos Biotechnology) on their surfaces before HFD feeding. Robust anti-IL-1α and anti-IL-1β auto-antibody responses were assessed by ELISA. Liver and fat tissue was analysed and RNA was isolated to assess gene expression via qPCR. **Results:** Our recent findings show that inactivation of IL-1 by vaccination with virus-like particles strikingly reduces steatosis in mice fed a HFD for 6 weeks on a macroscopic and molecular level. Lipid accumulation is drastically reduced and lipogenic enzyme as well as pro-inflammatory molecule expression is significantly downregulated in hepatic tissue. Although no cytokine expression is detectable in the liver at such an early time-point, inflammation and leukocyte infiltration is evident in adipose tissue. IL-1β serum levels show no differences between chow- or HFD-fed mice, pointing towards an indirect effect of fatty tissue inflammation on the liver. MK-2 deficient mice show no significant differences in liver weight or expression of any inflammatory markers in qPCR after 8 weeks of feeding. **Conclusion:** Our results strongly support future studies on IL-1 for clinical applications since its inactivation effectively prevents liver steatosis in HFD-fed mice. The present study indicates that the main initial target site for IL-1 is the adipose tissue rather than the liver itself. Therefore, the effect on the liver appears to be indirect as IL-1β serum levels do not increase upon feeding mice a HFD. Furthermore, MK-2 deficient mice fed either standard chow or HFD for eight weeks show no difference in liver or adipose tissue inflammation, indicating that the IL-1/IL-6 cross-talk does not affect the early phase of steatosis.

2.7 Bile acid-modulated transcript-expression in human macrophages validated by transcriptome analysis

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Cholestatic conditions are known to be associated with impaired innate and adaptive immunity, including monocytes, macrophages and T cells function. Recently, we demonstrated that bile acids modulate LPS-induced cytokine expression in primary human macrophages. Bile acids suppress LPS-induced inflammatory cytokine expressions. Moreover, bile acids adapt the activated macrophages to regulatory macrophages, characterized by an increased IL-10/IL-12 ratio. We hypothesize that bile acid influence the expression of genes, which generate the characteristic regulatory macrophages phenotype. Based on this result, an affymetrix-based mRNA transcriptomic analysis of LPS-stimulated macrophages, treated with bile acid was implemented. More than 12340 transcripts are differentially expressed in LPS-induced macrophages. Macrophages additional treated with bile acid lead to differentially expression of 1053 transcripts compared to LPS-stimulated macrophages alone. Interestingly out of this group, ~80 differentially expressed transcripts are associated with the immune system. First results demonstrated that bile acids reduce the LPS-induced expression of chemokines like CXCL1, CXCL3, CXCL9 and CXCL10 on mRNA and protein level. These regulate neutrophils and natural killer-cell migration. Additionally, chemokines like CCL3, CCL4 and CCL5 are declined by bile acids which are known to attract monocytes, dendritic-cells and neutrophils. Furthermore, CCL3, CCL4 and CCL5 play an important role for optimal CD8+ T-cell and antigen presenting cells interaction. Beside chemokines bile acids modify other LPS-induced transcripts that are associated with cell-cell contact (e.g. CD80, ICAM). These results lead to the assumption that bile acids affect the recruitment of immune cells to the side of infection by reducing the chemokine expression in human macrophages. Also, the cell-cell interaction and adhesion between macrophages and other immune cells could be influenced by bile acids. This assumption will be clarified in further investigations, e.g. co-culture experiments.

2.8 Binding Mode Prediction and Validation of Bile Acid and Neurosteroid Agonists of TGR5

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TGR5 is the first bile acid sensing G-protein coupled bile acid receptor (GPCR) GPCR and directly interacts with several G-protein subtypes [1]. High expression levels of TGR5 are found in the brain, the liver, and the gastrointestinal tract. TGR5 is an emerging target for the treatment of metabolic diseases [2 – 4]. Therefore, developing selective and potent agonists of TGR5 is of high importance [5]. However, without an x-ray crystal structure or an experimentally determined binding mode, the rational design of compounds is difficult. Here, we present an experimentally validated binding mode of 68 natural and synthetic bile acids and neurosteroids with agonistic activity towards TGR5. Our strategy includes multi-template homology modeling, molecular docking, and structure-based 3D-QSAR with subsequent mutational analysis and molecular dynamics simulations. After application of this strategy, our binding mode model of the 68 TGR5 agonists results in a good 3D-QSAR model ($q^2 = 0.50$), thus indicating that differences in the agonist structures correlate with differences in experimentally determined pEC50 values. Subsequently, nine mutants of binding site residues were suggested for experimental validation of the binding mode. Activity analysis with functional read-out and FACS analysis for membrane localization confirmed these predictions in all cases. Hydrogen bonding between Y240 and TGR5 agonists play an important role, which could be shown by a severe impairment of receptor activity upon Y240F mutation. Additionally, we identified the epimer selectivity determining residue Y89 for hydroxyl-groups in position seven on the cholane scaffold. This provides strong support to the validity of the binding mode. Through this, our binding mode could ease the structure-based design of new TGR5 agonists. We are grateful to the "Zentrum für Informations und Medientechnologie" (ZIM) at the Heinrich Heine University for computational support, Stefanie Lindner und Waltraud Kuß for technical assistance, and to Dr. Nadine Homeyer, Yasemin Bilgic, and Alina Völz for help with the molecular modeling. This work was supported by the Deutsche Forschungsgemeinschaft through the Collaborative Research Center SFB 974 ("Communication and Systems Relevance during Liver Damage and Regeneration", Düsseldorf) and the Clinical Research Group KFO 217 ("Hepatobiliary Transport in Health and Disease", Düsseldorf). [1] M. Hannah, Int. J. Infereron Cytokine Mediator Res., 2014, 6: 27 – 38. [2] T. W. H. Pols, et al., Cell Metab., 2011, 14: 747 – 757. [3] T. W. H. Pols, et al., J. Hepatol., 2011, 54: 1263 – 1272. [4] A. Perino, et al., J. Clin. Invest., 2014, 124: 5424 – 5436. [5] H. Sato, et al., J. Med. Chem., 2008, 51: 1831 – 1841.

2.9 Functional role of CCL5/RANTES for HCC progression during chronic liver disease: from humans to mice

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Background & Aims: Hepatocellular carcinoma (HCC) which is the consequence of malignant transformation of hepatocytes, is often based on chronic inflammation, resulting in liver fibrogenesis. During this process chemokines play a crucial role by attracting immune cells. In the current study, the function of CCL5/RANTES during chronic liver disease (CLD) was analysed in patients and two independent murine models of inflammation triggered HCC development. We showed that CCL5 acts mainly through immune cells by influencing the pro-inflammatory milieu in the diseased liver. Finally, we present an efficient therapeutic option for CLD in mice with implications for humans. **Methods:** The expression of CCL5 and its receptors was studied in well-defined CLD patients and in the two murine inflammation based HCC models $NEMO^{\Delta hepa}$ and $Mdr2^{-/-}$. The role of CCL5 in inflammation, fibrosis, tumour initiation and progression was analysed in different cell populations using $NEMO^{\Delta hepa}/CCL5^{-/-}$ mice and subsequent bone marrow transplantation (BMT) studies. For therapeutic intervention Evasin-4 was injected either for 24 hours or for 8 weeks. **Results:** In CLD patients, CCL5 expression correlated with inflammation stage and fibrosis grade in CLD patients. Interestingly, despite CCR1 and CCR3, CCR5 was upregulated in this cohort. This expression pattern was further confirmed in $NEMO^{\Delta hepa}$ mice during hepatocarcinogenesis. In contrast, in the second model of $Mdr2^{-/-}$ mice, only CCL5 was significantly upregulated, indicating a predominate role of this chemokine. Genetic inactivation of CCL5 in $NEMO^{\Delta hepa}$ mice diminished hepatocyte apoptosis, compensatory proliferation and fibrogenesis due to reduced immune cell infiltration. In this context, especially $CD45^+$ $Ly6G^+$ granulocytes, $CD45^+$ $CD11b^+$ $Gr1.1^+$ $F4/80^+$ pro-inflammatory monocytes, and T-$CD4^+$ - and $CD8^+$ cells were decreased in $NEMO^{\Delta hepa}/CCL5^{-/-}$ livers. As a consequence late stage $NEMO^{\Delta hepa}/CCL5^{-/-}$, mice displayed smaller and less malignant tumours, characterized by significantly reduced proliferative capacity and less pronounced angiogenesis in comparison with $NEMO^{\Delta hepa}$ tumors. Mechanistically, we identified hematopoietic cells as main source of CCL5, while CCL5 deficiency did not sensitise $NEMO^{\Delta hepa}$ hepatocytes towards TNF induced apoptosis. In a short term intervention experiment we demonstrate that especially $CD45^+$ $Ly6G^+$ granulocytes were reduced after Evasin-4 treatment. Subsequently, $NEMO^{\Delta hepa}$ mice were treated with Evasin-4 for 8 weeks. Pharmacologic intervention of the CCL5 pathway histologically resulted in a significant improvement of liver fibrosis confirmed by reduction of established fibrosis markers (αSMA, Collagen IA1, TGF-β, MMP-2 und MMP-3). **Conclusion:** Our present study identifies the essential role of CCL5 for disease progression and especially HCC development in men and in mice. Finally, inhibition of CCL5 *in vivo* appears to be encouraging for therapy of human CLD.

2.10 Changes in lipid and carbohydrate metabolism under mTOR- and calcineurin-based immunosuppressive regimen in adult patients after liver transplantation

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Background: Cardiovascular disease is a leading cause of long-term mortality after liver transplantation (LT). Life long immunosuppression harbours the risk of metabolic alterations. We aimed to analyze the impact of calcineurin (CNI)-only containing regimen (group A) compared to mTOR-containing regimen (group B) on lipid and carbohydrate metabolism. Patients/methods: 92 adult patients after LT, University of Mainz (group A - 78 patients, group B - 14 patients; 65 M/27F; mean age 59+/-10.2 ys; mean time from LT 5.8+/-5 ys). Clinical data, comorbidities, medication were assessed. Fasting lipid profile including small dense LDLs (sdLDL) and oral glucose tolerance tests were performed. **Results:** Group B had significantly higher levels of total cholesterol (TC), LDL-cholesterol (LDL-C), triglycerides (TG) and sdLDL, with persistence of higher TC, TG, sdLDLs (mg/dl) after exclusion of patients under lipid lowering medication. Concentrations above the upper limits of normal were found: for LDL-C in 9% of group A/35.7% of group B ($p = 0.016$); for TG: in 32.1% of group A/92.9% in group B ($p = 0.0001$). A positive correlation between time since LT (years) and sdLDL (mg/dl) was found in group B ($p = 0.018$). In patients without previously known diabetes, NODAT and impaired glucose tolerance developed in 27.9% of group A/44.4% of group B (n. s.). **Conclusion:** Patients under mTOR-containing regimen are at higher risk to develop dyslipidemia with increased atherogenic sdLDLs compared to patients under CNI-only-containing regimen and display more frequently a dysglycemic status, with uncertain relevance for long-term cardiovascular risk. A careful monitoring after LT is needed to identify early metabolic risk and manage this appropriately.

2.11 Combined effects of the prosteatotic TM6SF2 and PNPLA3 variants on severity of NALFD: multicentre biopsy-based study in German patients

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Introduction: The PNPLA3 (adiponutrin) mutation p.I148 M represents the common genetic risk factor for (non-)alcoholic fatty liver disease (NAFLD) and progressive liver fibrosis. Lately a second prosteatotic variant, TM6SF2 p.E167K, has been detected (Nat Genet 2014). So far it remains unclear if this mutation, alike the PNPLA3 p.I148 M variant, increases the risk of liver fibrosis. In the current project we analyse the combined effects of these variants on severity of NAFLD in a cohort of patients recruited at eight German tertiary referral centres. **Patients and Methods:** After exclusion of acute or chronic liver diseases other than NAFLD, 514 patients (age 16 – 88 years, 239 men) were included. In 309 patients liver biopsies were performed, which were examined by pathologists blinded to the genotyping results. PCR-based assays were used to genotype the PNPLA3 (rs738409) and TM6SF2 (rs58542926) variants. **Results:** The genotype frequencies of the PNPLA3 p.I148 M ([CC]= 215, [CG]= 223, [GG]= 76) and the TM6SF2 p.E167K ([CC]= 411, [CT]= 94, [TT] = 9) variants did not deviate from Hardy-Weinberg equilibrium. One copy of the prosteatotic PNPLA3 and TM6SF2 alleles was detected in 58% and 20% of NAFLD patients, respectively. Patients carrying the PNPLA3 p.I148 M or TM6SF2 p.E167K risk alleles had significantly (both $P < 0.01$) increased serum ALT and AST activities. The PNPLA3 risk genotype [GG] (OR = 2.15, $P = 0.007$), but not the TM6SF2 genotype was more frequent in individuals scheduled for liver biopsy. Among biopsied individuals, a total of 149 presented with hepatic steatosis grades S2 or S3, and fibrosis stage > 1 was detected in 77 patients. The TM6SF2 variant increased the risk of developing steatosis grades S2 – S3 (OR = 1.52, $P = 0.04$) but did not affect fibrosis stage. The PNPLA3 genotype was, in turn, associated with both steatosis grades S2 – S3 (OR = 1.94, $P < 0.001$) and increased fibrosis (OR = 2.24, $P < 0.001$). In patients with the PNPLA3 genotype [GG], the presence of variant TM6SF2 further increased serum aminotransferase activities (both $P < 0.05$) and tended to aggravate hepatic steatosis ($P = 0.09$). **Conclusions:** The PNPLA3 and TM6SF2 variants are associated with increased liver injury as reflected by serum surrogate and histopathological markers. The TM6SF2 polymorphism seems to modulate predominantly hepatic fat accumulation, whereas the PNPLA3 mutation confers risk of increased steatosis and fibrosis.

2.12 Procholestatic gene variants and mutations in secondary sclerosing cholangitis in critically ill patients (SC-CIP)

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Background and Aim: Secondary sclerosing cholangitis in critically ill patients (SC-CIP) has been recently defined as new disease entity. Its pathogenesis is unclear but ischemia and "toxic bile" might be involved in the development of SC-CIP. It was our aim now to study selected procholestatic gene variants and mutations in patients who developed SSC after an ICU stay. **Methods:** In total, we screened data of 4,641 cholangiography procedures performed between January 2008 and April 2015 at Saarland University Medical Center. In total, we identified 17 patients (age 33 – 80 years, 14 males) with a history of ICU treatment and signs of SSC. In these patients, we retrospectively analyzed the data concerning ICU therapy. Hepatic fibrosis was quantified with transient elastography. In all patients we genotyped the following procholestatic mutations (marked with an asterisk) and polymorphisms: ATP8B1 (p.N45T*, p.E429A*, p.I661T*, p.R952Q), ABCB4 (p.R590Q*, c.787A>T, c.504T>C), ABCB11 (p.E297G*, p.A444V, p.D482G*, c.3084A>G), and FXR (c.-1 G>T). The genotype frequencies were compared with data from reference populations. **Results:** Among the SC-CIP patients, myocardial infarction (n = 5, 29%) was the most common reason for the ICU stay, followed by cardiac surgery, pneumonia and polytrauma (n = 3 each). Median ICU stay was 32 days (range 8 – 167 days), and median duration of mechanical ventilation was 19 days (range 4 – 147 days). The first ERCP procedure was performed on average 105 days (range 24 – 1155 days) after the ICU treatment. All patients presented with increased cholestatic markers (median bilirubin 3.5 mg/dl, gamma-GT 731 U/l, AP 428 U/L), and most displayed markedly increased liver stiffness (median 22.6 kPa, range 3.5 – 75 kPa). All SC-CIP patients had infectious complications during their ICU stay. In 6 of 8 patients with cholangitis, microbiological analysis of bile revealed Entercoccus faecium (66.7%) and Enterococcus faecalis (50.0%) species; a single patient tested positive for Candida species. One patient was a heterozygous carrier of the FXR c.-1 g>t mutation. Otherwise, we did not detect any major differences in ABCB4, ABCB11 and ATP8B1 genotype distributions as compared to the general population. **Conclusions:** To our knowledge this is the first study investigating hepatobiliary transporter variants in patients with SC-CIP. Although our preliminary results do not indicate an association between the gene variants and SC-CIP, a more comprehensive approach (next-generation sequencing) is required to fully explore the impact of rare gene variants on SC-CIP risk.

2.13 Comparative induction of pluripotency in human umbilical vein endothelial cells and dermal fibroblasts and further differentiation into Hepatocytes

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Generating induced pluripotent stem cells (iPSCs) is a standardized technique. To date iPS cells have been generated from HUVECs using viral-based approaches. We employ an episomal plasmid-based approach to generate integration-free iPSCs (E-iPSCs) from human somatic cells. In this study, we compared the generation of E-iPSC lines from human umbilical vein endothelial cells (HUVEC) and fetal foreskin derived fibroblast (HFF1). The efficiency of inducing pluripotency in HFF1 was 0.03% compared to 2.5% in HUVEC. This implies that the efficiency of reprogramming HUVECs is 83-fold higher than in HFF1 cells. Additionally, the kinetics of reprogramming was much faster using HUVECs, i.e. three weeks for the stabilization of E-iPSC colonies compared to HFF1 cells which needed four months. The E-iPSCs from both somatic cell types were fully characterized and are comparable to human embryonic stem cells (hESCs). Both E-iPSC lines express pluripotency associated transcription factors as OCT4, NANOG, SOX2 and also the surface markers SSEA-4, TRA-l-60, TRA-1 – 81 and TRA-2 – 49 but not SSEA-1. Additionally, they have the ability to differentiate to all cell types representative of the three germ layers endoderm, ectoderm and mesoderm in vitro (by formation of embryoid bodies) and in vivo (teratoma formation in immunodeficient mice). We have already shown that E-iPSCs generated from HFF1 cells could be used to study hepatogenesis and represent a tool for studying human gastrulation at the molecular and cellular levels in vitro. We now demonstrate that HUVEC-derived E-iPSCs can be used to study hepatogenesis. A careful comparative analysis to determine which cell type would be better for generating hepatocyte-like cells with a more mature phenotype is in progress.

2.14 Der duale CCR2/CCR5 Antagonist Cenicriviroc reduziert die Infiltration pro-inflammatorischer CCR2+ Monozyten in Mausmodellen der akuten Leberschädigung

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Hintergrund: Das akute Leberversagen ist ein lebensbedrohliches Krankheitsbild mit begrenzten therapeutischen Optionen. Die Rekrutierung proinflammatorischer Monozyten über den CCL2-CCR2 Chemokin Signalweg in der akuten Leberentzündung stellt potentiell einen neuen Ansatzpunkt für Therapien dar. Die in die Leber eingewanderten Monozyten reifen zu Makrophagen (Mo-Mφ) aus, die sich deutlich von den residenten hepatischen Makrophagen (Kupffer-Zellen) unterscheiden. Um die Rolle der Mo-Mφ in der Phase der akuten Leberschädigung besser zu verstehen, haben wir den oralen CCR2/CCR5 Antagonisten Cenicriviroc (CVC), der sich aktuell in der klinischen Phase 2b zur Therapie von NASH und Leberfibrose befindet, in zwei Mausmodellen untersucht. **Methoden:** Zur Induktion des akuten Leberschadens wurden C57BL/6J und CCR2 defiziente Mäuse entweder mit CCl4 (0,6 mg/kg i.p.) oder Paracetamol (APAP, 250 mg/kg i.v.) behandelt. Anschließend erhielten die Mäuse in beiden Ansätzen oral entweder CVC (100 mg/kg) oder Vehikel (Vhc). Nachfolgend wurden der Leberschaden und immunologische Phänotyp der Gruppen untersucht. Zusätzlich wurden sowohl Mo-Mφ als auch residente Kupffer-Zellen im FACS isoliert und deren Genexpression mittels Array-basierter Nanostringanalyse untersucht. **Ergebnisse:** Sowohl die Applikation von CCl4 als auch von APAP führen zu einer schnellen und massiven CCR2 vermittelten Anreicherung proinflammatorischer Ly-6C+ Mo-Mφ in der Leber. In beiden Schadensmodellen reduziert die orale CVC Gabe Ly6C+ Monozyten sowohl im Blut ($p < 0,01$) als auch Ly-6C+ Mo-Mφ in der geschädigten Leber ($p < 0,001$). Die CVC vermittelte Inhibition der Monozyteninfiltration in die Leber korrelierte dabei ebenso mit einer signifikanten Reduktion der Serum-Transaminasen wie auch der Nekroseareale, sowohl im CCl4 vermittelten Schaden ($p < 0,01$) als auch im APAP induzierten Leberschadens ($p < 0,01$). Darüber hinaus lassen Nanostring-Genanalysen aufgereinigter Makrophagen-Populationen aus der Leber erkennen, dass im Gegensatz zu Ly-6C-, in Ly-6C+ Mφ Chemokine, Chemokin- und Toll-like-Rezeptoren heraufreguliert werden. Zusätzlich konnte gezeigt werden, dass der adoptive Transfer von aus dem Knochenmark stammenden Monozyten den APAP induzierten Leberschaden signifikant verstärkt, was den proinflammatorischen Phänotyp von CCR2+ Monozyten im akuten Leberschaden bekräftigt. **Schlussfolgerungen:** In unterschiedlichen Mausmodellen des akuten Leberschadens inhibiert CVC effektiv die Infiltration proinflammatorischer Monozyten in die Leber. CVC stellt somit eine vielversprechende, therapeutische Option zur Behandlung des akuten toxischen Leberversagens z.B. nach Paracetamolüberdosis, dar.

2.15 Development of De Novo Donor-specific Antibodies after Liver Transplantation with Antibody-mediated Rejection and Successful Treatment by Plasmapheresis

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Background: Although donor-specific antibodies (DSAs) are known to be an important risk factor in most solid organ transplantations, leading to increased risk of humoral rejection, the role of DSAs in liver transplantation is still not clarified. Liver transplantation was thought to play an exceptional role due to tolerogenic characteristics of the liver, but this point of view is questioned in recent years, since associations of DSAs and lower transplantation outcome were frequently reported. We report on a patient with DSA-positive humoral rejection after liver transplantation. **Methods:** Detection of DSAs was performed by Luminex mixed antigen beads assay and subsequent precise antibody testing by Luminex single antigen beads assay. Strength of DSAs was measured by mean fluorescence intensity (MFI). Case presentation: We present a case of a 54-year-old patient who developed de novo DSAs detected 18 months after liver transplantation, leading to severe hepatopathy with rapidly increasing transaminases and total bilirubin up to 19 mg/dl. The histological findings were inconclusive with chronic cholestasis, but without positive C4d-staining or chronic ductopenic rejection. Eight courses of Plasmapheresis were performed after detection of DSAs, resulting in sustained amelioration of patient's condition and decrease of bilirubin and transaminases. **Conclusion:** De novo donor-specific antibodies may well be responsible for graft failure after liver transplantation. Thus, respective diagnostic procedures for DSAs are recommendable, although the process of diagnosing might be difficult due to unspecific histologic criteria. Plasmapheresis is an efficient therapeutic procedure in case of DSA-associated graft failure.

2.16 Die Bedeutung des renalen Resistance-Index für das Nierenversagen nach den neuen Kriterien bei Leberzirrhose

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Einleitung: Nierenfunktionsstörungen bei Leberzirrhose sind häufig. Kürzlich wurden Kriterien für das Nierenversagen bei Leberzirrhose nach den AKIN-Kriterien evaluiert (Fagundes et al. J Hepatol 2013). Ein Anstieg des Serumkreatinins > 0,3 mg/dl innerhalb von 48 h bei einem Ausgangswert < 1,5 mg/dl wird abweichend von den bekannten Kriterien des hepatorenalen Syndroms (Voraussetzung: Kreatinin > 1,5 mg/dl) bereits als Nierenfunktionsstörung AKIN Grad I definiert. Ziel unserer prospektiven Studie war es zu untersuchen, ob die Bestimmung des renalen Resistance-Index (RI) bei Patienten mit einem Kreatininwert < 1,5 mg/dl am Tag der stationären Aufnahme eine Vorhersage für eine Nierenfunktionsstörung AKIN Grad I liefern kann. Götzberger et al. (Digestion 2012) berichteten, dass ein renaler RI-Wert > 0,74 mit dem Auftreten eines hepatorenalen Syndroms (nach den „alten" Kriterien) assoziiert ist. Studienaufbau: Wir haben 100 Patienten mit Leberzirrhose, die stationär aufgenommen wurden, untersucht. Am Aufnahmetag erfolgte zusätzlich zurer Ultraschalluntersuchung der Nieren eine standardisierte Messung der renalen RI-Werte. Ferner wurde Diagnostik bezüglich des Schweregrades der Leberzirrhose (MELD, Child-Score) und Infektionen (Urinstatus/-sediment, bei Aszites: Ausschluss SBP, bei V.a. Pneumonie Röntgen-

Thorax) durchgeführt und der Aszites beurteilt (Sonografie, Diuretikasensibel/-refraktär). Nach 48 h wurde eine erneute Kreatinin-Bestimmung durchgeführt. Anhand dieser Daten teilten wir die Patienten nach den AKIN-Kriterien ein. **Ergebnis:** Von 100 Patienten (Child A/B/C 46/44/10) erfüllten 35% die AKIN-Kriterien. Es zeigte sich ein signifikanter (p = 0,002) Unterschied der RI-Werte zwischen den Patienten mit (0,79 ± 0,10) und ohne Nierenfunktionsstörung nach AKIN (0,71 ± 0,07). 12 von 35 Patienten (34%) erfüllten die Kriterien AKIN I mit einem initialen Kreatininwert < 1,5 mg/dl. In dieser Gruppe zeigte sich im Vergleich zu der Patientengruppe ohne Nierenfunktionsstörung (Kreatinin < 1,5 mg/dl auch 48 h später) ein signifikant (p = 0,002) höherer renaler RI-Wert am Aufnahmetag (AKIN I: 0,75 ± 0,06, ohne Nierenfunktionsstörung: 0,70 ± 0,06). **Diskussion:** Die „neue" Einteilung des Schweregrades der Nierenfunktionsstörung bei Leberzirrhose schließt auch Patienten mit einem initial normwertigen Kreatinin (< 1,5 mg/dl) ein (AKIN Grad I). Mit der frühen Bestimmung des renalen Resistance-Index steht ein valides und nicht-invasives Verfahren zur Identifizierung dieser „Risiko"-Patienten zur Verfügung.

2.17 Die Therapie mit Terlipressin und Humanalbumin ist auch bei Patienten mit hepatorenalem Syndrom Typ 2 effektiv

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Einleitung: Das hepatorenale Syndrom (HRS) ist unbehandelt mit einer sehr schlechten Prognose assoziiert. Es wird unterteilt in ein HRS Typ 1 mit rasch progredientem Verlust der Nierenfunktion und einen Typ 2, gekennzeichnet durch eine chronische Hydropie und erhöhte Nierenretentionswerte. Während für das HRS Typ 1 eine Behandlung mit Terlipressin und Humanalbumin als effektiv gilt, ist umstritten inwieweit eine solche Behandlung auch bei Patienten mit HRS Typ 2 wirksam ist. **Methodik:** Über einen Zeitraum von 22 Monaten wurden alle Patienten mit erstmalig diagnostizierter Episode eines HRS erfasst. Klinisch relevante Parameter wie HRS-Typ, Patienten- und Therapiecharakteristika, Ansprechen, Gesamtüberleben sowie dialyse- und transplantationsfreies Überleben wurden prospektiv erfasst und ausgewertet. **Ergebnisse:** Insgesamt wurden 80 Patienten mit Leberzirrhose und Erstdiagnose eines HRS über einen medianen Zeitraum von 76 Tagen beobachtet. Bei der Mehrzahl der Patienten lag ein fortgeschrittenes Zirrhose-Stadium zugrunde (Child-Pugh C: 67; 84%). Ein HRS Typ 1 war bei 29, ein HRS Typ 2 bei 51 Patienten zu diagnostizieren (36%; 64%). Zwischen beiden Subgruppen bestand bei Diagnosestellung kein Unterschied hinsichtlich der Patientencharakteristika Ätiologie, Child-Stadium sowie Hydropie- und Encephalopathie-Grad. Erwartungsgemäß lag die mediane Serum-Kreatinin-Konzentration bei Diagnosestellung eines HRS Typ 1 signifikant höher als bei Typ 2-Patienten (3,1 vs. 2,4 mg/dl; p = 0,0003). Ein komplettes oder partielles Ansprechen auf Terlipressin wurde in 38 bzw. 4 von 80 Patienten beobachtet (48%; 5%). Die Gesamt-Ansprechrate in der Gruppe der Patienten mit HRS Typ 2 unterschied sich dabei nicht signifikant von der Ansprechrate bei HRS Typ 1 (53% vs. 52%; p = 0,92). Gesamtüberleben sowie dialyse- und transplantationsfreies Überleben waren in beiden Subgruppen ähnlich. In der Gruppe der Patienten mit HRS Typ 2 war das Ansprechen auf Terlipressin mit einem längeren medianen Überleben assoziiert (p < 0,0001). **Schlussfolgerung:** Terlipressin in Kombination mit Humanalbumin ist auch bei Patienten mit HRS Typ 2 effektiv – in etwa der Hälfte der Fälle ist mit einem Ansprechen zu rechnen. Das Ansprechen auf die Behandlung ist dabei mit einem Überlebensvorteil assoziiert.

2.18 Differential regulation of G-protein coupled bile acid receptor (Gpbar-1) by Sp1/KLF5 family transcription factors

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TGR5 (Gpbar-1) is a G-protein coupled bile acid receptor, which is highly expressed in cholangiocytes, liver sinusoidal endothelial cells, liver macrophages and CD14 positive monocytes of peripheral blood. TGR5-/- mice show impaired liver regeneration after partial hepatectomy and the increased inflammation in liver disease models, thus emphasizing a protective role of TGR5 in liver. While TGR5 functions are being studied extensively, little is known about the transcriptional regulation of this receptor. Aim: To investigate the mechanism and transcriptional regulation of TGR5. **Methods:** The potential TGR5 promoter (-154/-79) was cloned into a pGL3 Luciferase expression vector. Luciferase gene expression was used to evaluate the effect of Sp1 and KLF5 on TGR5 expression by co-transfection. Binding of the two transcription factors to the promoter was verified by Chromatin Immunoprecipitation (ChIP). Immunoprecipitation was used to confirm the interaction of HDAC1 and 2 with Sp1. Transfection of KLF5 siRNA was used to verify the TGR5 transcriptional regulation. **Results:** KLF5 increased the luciferase gene expression in a concentration dependent manner. The effect was lost when the binding site for KLF5 was mutated. High expression of SP1 mediated a downregulation of TGR5 promoter luciferase activity, which was absent when mutated Sp1 with a defective DNA binding domain was co-transfected. ChIP analysis confirmed the binding of both transcription factors to the predicted TGR5 promoter region. Sp1 forms an inhibitory complex with HDAC1 and 2 at the site of binding there by downregulating the transcription of TGR5. Inhibition of casein kinase 2 mediated phosphorylation of HDAC proteins by apigenin resulted in a decreased complex formation with Sp1 there by loss of Sp1 mediated downregulation. TGR5 is differentially regulated by Sp1 in different carcinoma cell lines (colon carcinoma and cholangiocyte carcinoma). KLF5 siRNA decreased the mRNA expression of TGR5 in colon and cholangiocyte carcinoma cell lines. **Conclusion:** This study demonstrates that the transcription factor Sp1 downregulates whereas KLF5 upregulates the gene expression of TGR5 in vitro. Sp1 decreases the transcription of TGR5 by binding to the promoter region and recruiting HDAC1 and 2 to forming an inhibitory complex thereby leading to the deacetylation of histones. Phosphorylation of HDAC proteins by casein kinase 2 is important in the formation of inhibitory complex. Differential regulation of TGR5 by Sp1 was confirmed in different carcinoma cell lines.

2.19 Efficacy and safety of Boceprevir triple therapy in previously treated patients with HCV Genotype 1 (G1) infection in German real-life

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Background: Since 2011, triple therapy with the HCV protease inhibitor boceprevir (BOC) is widely used as standard of care for patients (pts) with chronic HCV G1 infection. The present interim analysis of the NOVUS observational study investigated the efficacy and safety of BOC triple therapy in pretreated patients in German real-world according to the previous virologic response to dual therapy with pegylated interferons (PegIFN) and ribavirin (RBV). **Methods:** From April 2012 until January 2014, 536 pts with G1 infection were recruited in the ongoing NOVUS study by 97 practices and hospitals in Germany. Pts were treated with PegIFN/RBV/BOC up to 44 weeks after a 4 weeks lead-in period with PegIFN/RBV. The present interim analysis was restricted to 138 pretreated pts who started triple therapy at least 12 months ago. **Results:** Pts distribution was 59% male, 60% aged > 50 years, 30% G1a, 51% G1b, 71% with viral load > 400,000 IU/mL, 10% cirrhotics, 9% under opioid substitution and 3% co-infected with HIV. Regarding previous response to dual therapy 38% were relapsers, 20% null-responders, 15% partial responders, 6% had breakthrough and 21% with unknown previous response. Based on available data from this ongoing study, SVR was achieved by 57% (62/108) in the total population. The highest SVR (86%, 42/49) was observed in pts who achieved an early virologic response (EVR) at treatment week 8 followed by pts with a > 1 log HCV-RNA decline at treatment week 4 (68%, 44/65). When pts were analyzed according to previous response, following SVR rates were obtained: Partial responders 65% (13/20), relapsers 60% (32/52), previous break-

through 50% (3/6) and previous unknown response 58% (11/19). The lowest SVR rate of 24% (5/21) was attained in the subgroup of previous null-responders with a high proportion (30%) of cirrhotic pts. Triple therapy was prematurely discontinued in overall 35% with the highest frequency of 71% in null-responders. Regarding adverse events, 36% and 5% showed Hb declines to > 8.5 – < 10.0 g/L and < 8.5 g/dL, respectively. 27% of pts developed exclusively elevated TSH levels indicative for hypothyroidism. In 8% of pts an eGFR decline to < 60 mL/min x 1.73 m2 was observed. All of these pts were older than 50 years and had renal insufficiency grade 2 at baseline. **Conclusions:** Previously treated pts undergoing BOC triple therapy for HCV G1 infection in German real-world attained an overall SVR rate of 57% which was highest (86%) in pts who achieved an EVR. In contrast, a low SVR rate of only 24% was achieved by previous null-responders.

2.20 Pre-existing co-morbidities and co-medications of patients undergoing treatment of chronic HCV G1 infection in German real-life

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Background: Information about co-morbidities of patients (pts) currently treated for chronic HCV genotype 1 (G1) infection in real-life is scarce. The present interim analysis of the NOVUS observational study was therefore aimed to investigate the frequency of pre-existing and ongoing co-morbidities of pts treated for chronic HCV G1 infection in German real-life and to determine the frequency of co-medications. **Methods:** From April 2012 until January 2014, 536 pts with HCV G1 infection were recruited in the ongoing NOVUS study by 97 practices and hospitals in Germany. Until now, pre-existing co-morbidities before triple therapy of HCV G1 infection with boceprevir (BOC) were documented for 469 pts. **Results:** Ongoing co-morbidities were reported for 329 of 469 pts (70%) before treatment of HCV G1 infection. Overall, 599 ongoing co-morbidities (multiple answers allowed) were documented. The most frequently reported co-morbidities were obesity (BMI > 30 kg/m2) (19%), cardiovascular diseases (18%), psychiatric disorders (14%), opiate substitution (13%), gastrointestinal diseases (11%), metabolic disorders (8%), thyroid gland diseases (7%), bone and joint diseases (6%), skin diseases (5%), HIV-co-infection (4%) and kidney diseases (2%). When co-morbidities were analyzed by gender (female vs. male), thyroid diseases occurred more frequently in females (13% vs. 3%, P < 0.0001), while opiate substitution (9% vs. 15%, P < 0.04) and HIV- co-infection (1% vs. 6%, P < 0.01) occurred less frequently in female pts. Regarding age (< 50 vs. > 50 years), cardiovascular (9% vs. 28%, P < 0.0001), thyroid (3% vs. 12%, P = 0.0002) and kidney diseases (0.4% vs. 5%, P = 0.0025) were more frequently reported in pts elder than 50 years, while opiate dependence (20% vs. 4%, P < 0.0001) and HIV-co-infections (6% vs. 1%) were less frequently reported in elder pts. Co-medications were documented for 233 of 469 pts (50%). According to the frequency of co-morbidities, 39% of overall 564 co-medications were related to treatment of neuropsychiatric disorders including opiate dependency, 19% were cardiovascular drugs, 14% were agents for gastrointestinal and metabolic diseases and 6% for thyroid diseases. 64 co-medications in 48 pts had the potential to interact with BOC. **Conclusions:** The present analysis demonstrates that preexisting co-morbidities are a frequent problem in pts undergoing treatment of HCV G1 infection in German real-life and that several co-morbidities are related to gender or age. As a consequence, there is a high frequency of co-medications which needs attention with regard to possible interactions with direct-acting antivirals.

2.21 Treatment of chronic HCV Genotype 1 infection with Boceprevir in German real-life:The impact of SVR on initially elevated and initially normal serum alanine aminotransferase (ALT) and gamma-glutamyl transpeptidase (GGT) levels

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Background: Patients (pts) with chronic HCV G1 infection may present with normal or elevated serum ALT or GGT levels. The aim of the present analysis was to investigate the effect of successful treatment of chronic HCV G1 infection with boceprevir (BOC) triple therapy on initially elevated and initially normal ALT and GGT levels. **Methods:** From April 2012 until January 2014, 536 pts with HCV G1 infection were recruited in the ongoing NOVUS observational study by 97 practices and hospitals in Germany. Pts were treated with pegylated interferons (PegIFN) and ribavirin (RBV) together with BOC for 24 to 44 weeks after a 4 weeks lead-in period with PegIFN/RBV. The present interim analysis was restricted to 144 treatment-naïve pts and 60 previously treated pts who achieved a sustained virologic response (SVR) 24 weeks after termination of HCV treatment. **Results:** 80% (115/144) and 40% (56/139) of treatment-naive pts presented with elevated ALT and GGT levels before HCV therapy. Compared to treatment-naïve pts, a similar frequency of ALT elevations (72%; 43/60) was found in previously treated pts while GGT elevations occurred more frequently (59%; 34/58; P = 0.019). Achieving SVR resulted in a normalization of initially elevated ALT and GGT levels in 94% and 91% of treatment-naive pts 24 weeks after termination of triple therapy. Comparable and statistically not different results were observed in previously treated pts with SVR who achieved normalization of ALT and AST in 95% and 96%, respectively. In treatment-naïve pts with normal serum ALT and GGT levels at baseline achievement of SVR was associated with a significant (P < 0.0001) ALT and GGT decline by 33% and 38%. Similar results showing a significant ALT and GGT decline by 49% (P < 0.0001) and 36% (P = 0.0005) were obtained in previously treated pts with initially normal ALT and GGT levels. **Conclusions:** Achievement of SVR following treatment of chronic HCV G1 infection with BOC in German real-world is accompanied by a normalization of initially elevated ALT and GGT levels in more than 90% of previously untreated and previously treated pts. In addition, achievement of SVR results in a significant decline of initially normal ALT and GGT values. Whether this decline reflects reduction of liver cell injury despite initially normal ALT and GGT levels remains to be investigated.

2.22 Boceprevir triple therapy of chronic HCV genotype 1 (G1) infection in previously untreated patients: Efficacy, predictability of virologic response and safety in German real-life

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Background: Since 2011, triple therapy with the HCV protease inhibitor boceprevir (BOC) is widely used as standard of care for patients (pts) with chronic HCV G1 infection. Here we report the German experience obtained from the NOVUS observational study with respect to efficacy, safety and predictability of virologic outcome in previously untreated pts undergoing BOC triple therapy in real-life. **Methods:** From April

2012 until January 2014, 536 pts with G1 infection were recruited in the ongoing NOVUS study by 97 practices and hospitals in Germany. Pts were treated with pegylated interferons (PegIFN) and ribavirin (RBV) together with BOC for 24 to 44 weeks after a 4 weeks lead-in period with PegIFN/RBV. The present interim analysis was restricted to 275 treatment-naïve pts. **Results:** Pts distribution was 59% male, 39% aged > 50 years, 37% G1a, 49% G1b, 69% with viral load > 400,000 IU/mL, 5% cirrhotics, 13% under opioid substitution and 4% co-infected with HIV. At treatment week (TW) 8, 72% (173/241) achieved an early virologic response (EVR) which allows shortening of triple therapy to 24 weeks. Until now, SVR was attained by 76% (151/199) in the total population and 87% vs. 46% in pts with/without EVR (p < 0.0001), respectively. Significantly different EVR and SVR rates in various subgroups are summarized in the table. Multivariate stepwise logistic regression analysis identified firstly HCV-RNA ≤ 400,000 IU/mL (OR = 2.6, P = 0.01), normal GGT levels (OR = 4.0, P < 0.0001) and a HCV-RNA decline > 1log10 at TW4 (OR = 6.9, P < 0.0001) as independent predictors of a high EVR rate and, secondly, normal GGT (OR = 2.9, P = 0.012), age < 50 years (OR = 3.4, P = 0.0009) and HCV-RNA decline > 1log10 at TW4 (OR = 5.6, P = 0.0001) as independent predictors of a high SVR rate. Regarding adverse events, 17% developed thyroid dysfunctions, 4% showed eGFR declines to < 60 mL/min x1.73m2 while 29% and 8% developed moderate (Hb > 8.5 – < 10 g/dL) and severe anemia (Hb< 8.5 g/dL), respectively. Anemia was managed by RBV dose reductions in 64% while 7% received blood transfusions. Only 1 patient was treated with erythropoietin and no pts discontinued triple therapy because of anemia. Regarding achievement of SVR, there were no differences between pts with and without anemia (74% vs. 77%, P = 0.56). **Conclusions:** Following treatment of HCV G1 infection with BOC triple therapy in German real-life, 76% of previously untreated pts achieve a SVR. 72% of pts attain an EVR which is associated with a SVR rate of 87%. Normal GGT values at baseline, age < 50 years and a HCV-RNA decline > 1log10 at TW4 are independent predictors of higher EVR and SVR rates.

2.23 Heart rate variability and heart rate turbulence correlated with the complications and the progress of cirrhosis and might predict the outcome of cirrhotic patients

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Background: During liver cirrhosis cardiac dysfunction is frequent and is associated with morbidity and mortality. Cardiac dysfunction in cirrhosis is presented as systolic and diastolic dysfunction, structural and electrophysiological abnormalities. This study investigates the cardiac dysfunction using advanced holter diagnostic in patients with liver cirrhosis. **Methods:** Eighty-two (63% male) patients with liver cirrhosis received a standard 12 leads ECG and 24 h holter monitoring including heart rate turbulence (HRT) and heart rate variability (HRV). The median Child score was 6 (range 5 – 12) and median MELD was 10 (range 6 – 32) and the median age was 60.5 years (range 19 to 89). Furthermore, biochemical parameters were analyzed using standard methods. **Results:** Interestingly, increasing Child score and its parameters low albumin and high INR correlated with more severe abnormalities of parameters of HRV (SDANN; standard deviation of the averages of NN intervals) and HRT (TO; turbulence onset). In addition, patients with ascites, history of HE and HRS showed more abnormalities in the parameters of HRV and HRT. Moreover, lower Hb and hematocrit, as well as low sodium and high CRP correlated with severer pathologies of HRV and HRT parameters. During the observation period, patients with rising MELD score showed significantly more abnormal HRV and HRT parameters. **Conclusion:** Parameters HRV and HRT correlated with the degree and the complications of cirrhosis and might be useful to uncover cardiac dysfunction in cirrhosis.

2.24 Hepatitis B Reaktivierung unter Chemotherapie und Immunsuppression – eine monozentrische Studie an 4868 Patienten

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Hintergrund: Eine Hepatitis B (HBV)-Reaktivierung unter Immunsuppression (IST) oder Chemotherapie (CTX) ist eine vermeidbare Komplikation, die mit einer hohen Mortalität und Morbidität verbunden ist. Für Europa gibt es kaum Daten zu Reaktivierungshäufigkeit, Risikofaktoren und Outcome. **Methoden:** Retrospektive, monozentrische Analyse, 4868 Patienten mit CTX oder IST 08/2002 – 12/2011. Erfasst wurden u.a. CTX-Schema und Virologiebefunde. Bei HBV-Reaktivierung erfolgte eine detaillierte Analyse von Patientencharakteristika, Serologie, Management und Outcome. **Ergebnisse:** Von 4868 Patienten wurden 66,2% mindestens einmal auf HBV getestet, 8% davon waren anti-HBc positiv. 1% wies eine chronische HBV-Infektion (HBSAg und/oder HBV DNA positiv) auf, bei 6% davon trat ein Flare unter CTX auf. In 1% anti-HBc-only-Status, davon reaktivierten 14%. Ausgeheilte HBV-Infektion bei 6% der HBV-getesteten, 2% davon mit Reaktivierung. Insgesamt 22 Reaktivierungspatienten (0,75% der HBV-getesteten; 10% der anti-HBc positiven; Diagnose 57,5 ± 63,5 Monate nach Beginn der CTX). 19/22 Reaktivierungspatienten überlebten, 2 verstorben (1 Leber-assoziiert), ein Outcome unbekannt. HBV-Infektion vor Reaktivierungsdiagnose bei 17/22 bekannt, bei 2 Reaktivierungsprophylaxe indiziert, einer erhielt die Prophylaxe. 15/22 erhielten CTX (1 allogene/1 autologe Stammzelltransplantation), 7 IST (5 rheumatologische, 2 Autoimmun-Erkrankungen). 8/22 erhielten Rituximab, 7 Anthrazykline, 16 Kortikosteroide. 12/22 hatten eine Lymphozytopenie zum Reaktivierungs-Zeitpunkt. HBV-Reaktivierungstherapie mit Entecavir n = 5, Lamivudin n = 6, Tenofovir n = 1 Telbivudin n = 1, 3 dokumentierte Therapieempfehlungen, 5 ohne dokumentierte Therapie. Viruslast bei Reaktivierung 1,7E+03 (n = 22 HBV-DNA-positiv/n = 6 HBsAg-positiv, n = 15 HBsAg-negativ), negative HBV-DNA 6,4 ± 8,0 Monate nach Beginn der HBV-Reaktivierungstherapie. **Schlussfolgerung:** Die bisherige Datenanalyse zeigt, dass auch in Deutschland eine HBV-Reaktivierung ein zwar seltenes aber dennoch klinisch relevantes Problem darstellt. Alle Patienten sollten vor Beginn einer CTX oder IST ein HBV-Screening erhalten.

2.25 Hepatocyte-like cell platforms for in vitro evaluation of antisense drugs in familial amyloidosis using stem cell technology

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Familial amyloidosis (ATTR) is a neurodegenerative disease caused by mutations of the transthyretin (TTR) gene that is mostly (> 95%) expressed by liver. Currently, more than 100 TTR mutations have been discovered in patients with ATTR, with different clinical manifestation. Drug therapy of ATTR is partially possible on a protein level. However, a full silencing of the mutated TTR gene would enormously increase therapeutic outcome. Antisense oligonucleotides (ASOs) and small interfering RNAs (siRNAs) are the most commonly used gene-silencing strategies. These artificial nucleotides bind complementary to the target gene mRNA, hence inactivating the translation of the protein. In this study, ATTR patient-specific hepatocyte-like cells (iPSC-Heps) were generated from induced pluripotent stem (iPS) cells, followed by a broad in vitro analysis of ASOs and siRNA effects on TTR expression. Fresh urine (250 – 500 ml) from ATTR patients (n = 12) was processed for isolation of renal epithelial cells, followed by reprogramming into iPS cells by using integration-free vectors, such as nucleofection of episomal (EBNA) plasmids. After characterization of iPS colonies, a 3-step differentiation protocol toward hepatocytes was performed. Briefly, iPS cells encountered a treatment with specific growth factors (activin A, Wnt3a, FGF2, HGF) for 14 days. iPSC-Heps were then characterized by analysis of typical hepatic markers, e.g. albumin and TTR via RT-PCR, immunocytochemistry and hepatocyte-specific functional assays. For TTR gene silencing, ASOs or

siRNAs were introduced into iPSC-Heps using cationic lipid. The phenotype of TTR knockdown in iPSC-Heps was examined in vitro. After 2 weeks of cultivation of urinary cells, 4 – 6 stable cell populations emerged. Episomal vectors yielded iPSCs with characteristic features. The iPSC-Heps indicated a high similarity to primary human hepatocytes. ASO or siRNA treatment generated TTR depleted ATTR iPSC-Heps. Urine cell-derived iPSCs can be reprogrammed and efficiently differentiated to iPSC-Heps. Antisense drugs are suitable tools for TTR knockout in ATTR iPSC-Heps. As a result, ASOs or siRNAs could be potentially useful for pharmacological development and regenerative medicine.

2.26 Improved survival in patients with primary sclerosing cholangitis and normalization of serum cholestasis markers after biliary dilatation therapy

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Background & Aims: Primary sclerosing cholangitis (PSC) is aggravated by dominant stenosis of the large bile ducts (DS) in the majority of cases. PSC patients with concomitant DS require endoscopic dilatation therapy to resolve biliary obstruction, but survival still remains impaired. We aimed to analyze the advantage of serum cholestatic markers to predict dilatation- and transplantation-free survival in PSC patients after endoscopic therapy. **Methods:** We analyzed 131 PSC patients that were treated at our tertiary care center with regard to serum cholestatic marker after endoscopic dilatation therapy. Clinical and laboratory data was obtained by chart review. The end point was defined as liver transplantation or death. **Results:** Within one year after dilatation therapy 42/131 (32.1) patients reached normalization of ALP values, 69/131 (52.7) patients normalization of serum bilirubin levels and 51/131 (38.9) patients reached a normalization of serum GGT levels. 60/131 (45.9) patients developed re-stenosis, requiring again endoscopic dilatation therapy. Patients with improvement of ALP after first dilatation therapy developed significantly less re-stenosis (26.2 vs. 57.0; $p < 0.001$). The same was true for patients with improvement of GGT and reduction of all three cholestasis parameters (32.7 vs. 56.9; $p = 0.007$ and 30.6 vs. 53.3; $p = 0.021$, respectively). Re-dilatation free survival was significantly improved in patients with normalization of ALP (log-rank: 10.1 ± 1.5 vs. 5.2 ± 0.9; HR = 0.4; 95% CI, 0.2 – 0.8; $p = 0.013$) and normalization of all three cholestasis serum markers (log-rank: 9.7 ± 1.5 vs. 5.3 ± 0.9; HR 0.5; 95% CI 0.3 – 0.9; $p = 0.032$). Within the observational period 57/131 (43.5) patients reached the combined clinical end point. Transplantation-free survival after dilatation therapy was improved in patients with normalization of ALP, bilirubin and the combination of all three serum cholestasis marker (log-rank: ALP normalization: 11.8 ± 0.6 vs. 15.8 ± 3.2, $p = 0.029$; bilirubin normalization: 9.2 ± 0.8 vs. 14.4 ± 1.9, $p = 0.005$; normalization of ALP, bilirubin and GGT: 11.8 ± 0.5 vs. 15.8 ± 3.7, $p = 0.023$). In multivariate analysis MRS, presence of IBD as well as normalization of ALP, bilirubin and normalization of all three serum cholestasis marker were independently associated with transplantation-free survival after first endoscopic dilatation therapy. **Conclusions:** In conclusion we were able to identify serum cholestasis marker as independent risk factor for dilatation- and transplantation-free survival in patients with PSC and concomitant DS. Especially ALP might be an adequate parameter to develop rationale endoscopic surveillance strategies and to identify patients at risk for progressive liver disease.

2.27 In alcoholic cirrhosis, low serum hepcidin levels associate with poor long-term survival and higher occurrence of hepatocellular carcinoma

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Background & Aims: Iron constitutes a potentially toxic element and consequently, hepatic iron overload may accelerate liver disease progression and development of hepatocellular carcinoma (HCC). Hepcidin is the central negative regulator of iron metabolism that is produced primarily by the liver. **Methods:** To study the prognostic significance of serum hepcidin, we assessed the influence of baseline serum hepcidin levels on outcome of a French cohort encompassing 237 patients with alcoholic liver cirrhosis prospectively followed-up in the setting of HCC screening. **Results:** Hepcidin values correlated weakly with serum ferritin levels ($r = 0.33$) and hepatic iron scores ($r = 0.3$). After a median follow-up of 68 months, patients with baseline lower hepcidin level had a higher risk of HCC occurrence (HR = 1.75 [1.02 – 3.12], $P = 0.04$) and overall death (HR = 1.63 [1.09 – 2.43], $P = 0.01$). According to univariate analysis, lower hepcidin level was associated with overall death (Hazard ratio, HR = 1.63 [1.07 – 2.44] $P = 0.01$) along with higher liver iron score ($P = 0.02$), higher transferrin saturation ($P = 0.05$) older age ($P = 0.002$), higher Child-Pugh score ($P < 0.0001$), increased BMI ($P = 0.02$) and lower platelet count ($P = 0.09$). According to Cox multivariate analyses, lower hepcidin levels were independently associated with death (HR = 2.8 [1.3 – 6.3]; $P = 0.01$) along with higher Child-Pugh score while HCC occurrence was mainly associated with clinical confounders interfering with iron metabolism (older age and higher BMI). **Conclusion:** In conclusion, low serum hepcidin levels in patients with alcoholic cirrhosis bear a long-term prognostic significance warranting further explorations.

2.28 Inadequate vitamin D levels are not associated with dietary vitamin D intake in patients with chronic liver diseases but correlate with reduced light exposure as quantified by actigraphy

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Background: Patients with chronic liver diseases (CLD) commonly exhibit vitamin D deficiency due to both environmental and endogenous factors. The vitamin D receptor is expressed in immune and non-parenchymal liver cells and vitamin D is known to affect the defence against bacterial infections in CLD. The specific aim of this study was to assess the associations of exogenous factors such as dietary intake and sunlight exposure with serum vitamin D levels in CLD patients. **Patients and Methods:** Serum 25-hydroxyvitamin D concentrations were measured with chemiluminescence immunoassay in German patients with viral and non-viral CLD. We quantified light exposure using the accelerometer-based physical activity monitor ActiGraph GT3X with integrated ambient light sensor. Light intensity was reported as lux (lm/m2). Dietary intake was simultaneously captured using food diaries and analysed using EBISpro software. In addition, bioelectrical impedance analysis (BIA) determined body composition. **Results:** In total, we included 30 patients with a median age of 55 years (27 – 81), 63.3% were women, and median BMI was 26.9 kg/m2 (19.3 – 39.2). All patients displayed normal patterns of circadian activity. In all patients, serum vitamin D levels correlated significantly ($rs = 0.56$, $P = 0.02$) with light exposure. Significantly ($P = 0.009$) higher daily exposure to all thresholds of lux intensities were displayed in patients with normal vitamin D levels (> 30 ng/ml), as compared to those with vitamin D deficiency. Significantly higher lux was accrued in spring and summer as opposed to autumn and winter ($P < 0.0001$). Dietary vitamin D intake was lower (median 1.48 µg/day, 0.26 – 12.12) than the national average (2 – 4 µg) and did not correlate with serum vitamin D levels. Patients with liver cirrhosis tended to have lower light exposure ($P = 0.04$). Light intensity was the only independent predictor of serum vitamin D level in multivariate regression analysis ($P = 0.004$). **Conclusions:** In CLD patients, serum vitamin D levels positively correlate with light exposure but not with dietary vitamin D intake. The contribution of vitamin D from diet is minimal and below the recently revised recommendations for vitamin D intake. In contrast, our findings highlight the need for combined interventions to treat vitamin D deficiency and may guide specific recommendations for sunlight exposure in patients with CLD.

2.29 Six-month vitamin D replacement reduces hepatic steatosis in the absence of weight loss

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Introduction: Non-alcoholic fatty liver disease (NAFLD) is highly prevalent in Western countries, ranging from 25 to 45%. Recently decreased serum 25-hydroxyvitamin D levels in patients with NAFLD have been confirmed in a meta-analysis of observation studies. This intervention study investigates whether a reduction in hepatic steatosis occurs with vitamin D replacement in patients referred to an outpatient liver clinic. **Methods:** In total, 40 patients with NAFLD and vitamin D deficiency were prospectively recruited. Vitamin D deficiency was defined by serum 25-hydroxyvitamin D concentrations < 20 ng/ml and was measured using chemiluminescent immunoassay. Hepatic steatosis was assessed using vibration-controlled transient elastography (FibroScan) with controlled attenuation parameter (CAP). Patients with significant liver fat accumulation were included (defined by CAp ≥ 280 dB/m). All patients received 20,000 IU cholecalciferol daily for 7 days, then weekly for 6 months. During this time we monitored CAP values, vitamin D levels, liver function tests (LFTs), parathyroid hormone (PTH) and body composition (assessed using bioelectrical impedance analysis). **Results:** The cohort comprised 52.5% men (age 54.9 ± 12.1 years; BMI 29.5 ± 3.0 kg/m2), and mean vitamin D levels were 11.8 ± 4.8 ng/ml. Transient elastography revealed a mean baseline CAP value of 329.8 ± 31.7 dB/m. CAP scores significantly decreased (improved) during the 6-month supplementation period (P = 0.007, ANOVA). Specifically, a mean CAP reduction relative to baseline was demonstrated at 4 weeks as well as after 3 and 6 months: -5.3 ± 13.8%; -6.0 ± 14.6% and -6.4 ± 13.0%, respectively. A significant inverse correlation between CAP values at baseline and relative CAP change during the supplementation period was noted (r = -0.418, P = 0.02). The supplementation regimen successfully restored serum 25-hydroxyvitamin D concentrations to normal values at 4 weeks, and at 3 and 6 months (34.6 ± 12.9, 36.3 ± 10.2, 34.8 ± 9.8 ng/ml; P < 0.0001). No changes in body composition or serum LFTs were noted during these 6 months. **Conclusions:** Hepatic steatosis, as assessed using CAP, significantly improves with serum 25-hydroxyvitamin D restoration. Vitamin D levels return to normal levels after only 4 weeks of supplementation, which coincides with significant liver fat reduction. Hepatic steatosis is a dynamic process, which might be modulated by short-term therapeutic interventions such as vitamin D substitution.

2.30 Keratin 23 represents a novel liver injury marker reflecting the severity of ductular reaction

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Keratins (K) are the intermediate filaments of epithelial cells and constitute established diagnostic tools. In the liver, K7/K19 expression is restricted to hepatic progenitor cells (HPCs) and biliary epithelial cells (BECs). Consequently, K7/K19 represent a widely used marker of the regenerative liver response termed ductular reaction (DR) that consists of activated BECs and HPCs. **Methods:** Since K23 is a largely unknown keratin family member, we analysed its expression and localization in selected human liver disorders and mouse liver injury models using custom-made antibodies. Serum K23 levels were measured via dot blot analysis. K23 regulation in response to IL1b was studied in hepatocellular and bile duct carcinoma cell lines. **Results:** In untreated mice, K23 was found in biliary epithelia but not hepatocytes. It was (together with K7/K19) markedly up-regulated in three different DR/cholestatic injury models, i.e. MDR2 knockouts, animals treated with 3,5-diethoxycarbonyl-1,4-dihydrocollidine or subjected to bile duct ligation. No changes in K23 levels were seen in hepatocellular injury models such as partial hepatectomy or carbon tetrachloride-induced fibrogenesis. K23 levels correlated with the DR marker Fn14 and immunofluorescence staining showed a distinct but not perfect co-localization with K7/K19. In cell culture, K23 expression was upregulated after IL1b treatment. In humans, K23 levels were moderately up-regulated in active HCV (~3 times) and ALD (~10 times). K23 expression was higher in patients with more prominent inflammation/fibrosis. A dramatic increase (> 200 times) was observed in patients with ALF and end-stage PBC. K23 serum levels were significantly higher in patients with alcoholic liver cirrhosis compared to control subjects. In conclusion, K23 represents a novel, stress inducible DR marker and its levels correlate with the severity of the liver disease. Given its release into the serum and its rather specific expression pattern, it may represent an attractive non-invasive marker of liver injury/regeneration.

2.31 Microbubbles as used for contrast-enhanced ultrasound affect the migration of human primary leukocytes

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Poly n-butylcyanoacrylate (PBCA) microbubbles are used for a wide variety of biomedical applications, such as targeted delivery of drugs or as contrast agents for ultrasound imaging of the liver. Microbubbles are generally considered as non-toxic and immunologically inert. Up to now, possible effects of PBCA microbubbles on immune cells are unclear. Here, we studied the effects of the microspheres on human primary leukocytes with a focus on cell migration and differentiation. Microbubbles, which had been labeled with rhodamine for fluorescent detection, did not cause cytotoxic effects upon incubation with primary human leukocytes. Interestingly, microbubbles significantly inhibited the migration of lymphocytes and monocytes, whereas the migration of granulocytes and mature macrophages remained unaffected. Flow cytometric studies were performed to elucidate the cellular uptake of the microbubbles by immune cells, and demonstrated that microbubbles were primarily cleared by monocytes in a dose-dependent manner. This internalization was energy-dependent, since it was blocked upon incubation at 4 °C. Realtime-PCR revealed that microbubbles did not affect inflammatory mediator gene expression by human primary macrophages, even at up to seven days of incubation. Our findings demonstrate a cell-specific inhibition of migration of peripheral blood mononuclear cell subsets by the micro-sized constructs, indicating potential immunological side-effects of PBCA microbubbles in patients with inflammatory liver diseases.

2.32 Natural course and prognostic factors in patients with cholestatic liver disease – experience from a single center study

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Background and Aims: Primary biliary cholangitis (PBC) and primary sclerosing cholangitis (PSC) are the most common cholestatic liver diseases. However, the clinical course of PBC and PSC differs significantly among individuals and therefore scores using disease-specific parameters are necessary to identify patients who are at high risk developing complications with impaired prognosis. The aim of our study was to analyze the typical characteristics of patients with PSC and PBC focusing on treatment, prognostic factors and the prognostic significance of the recently established albumin-bilirubin (ALBI) score. **Methods:** 338 patients with PSC/PBC who were treated at our Liver Unit between 1996 and 2015 were included in the analyses. Medical records, laboratory parameters, treatment, transplant-free survival and overall survival (OS) were assessed. **Results:** 214 patients with PBC (89.3% female, median age 64 years) and 124 patients with PSC (67.7% male, median age 49 years) were included in the analyses. 71.8% of patients with PSC had concurrent IBD, especially ulcerative colitis (80.9%), compared to 1.4% of PBC patients. Significantly more patients with PSC developed colorectal neoplasia compared to PBC patients (11.3% vs. 1.4%). Patients with PSC showed higher ALBI score values than PBC patients (p < 0.001) indicating more severe liver disease. Bilirubin (HR 1.35; 95% CI: 1.14 – 1.61; p = 0.001) was the strongest significant negative prognostic factor for transplant-free survival. Adding albumin as a marker of liver function, the ALBI score significantly identifies patients with worse prognosis. **Conclusion:** The ALBI score can be used to assess liver function, disease severity and OS in patients with cholestatic liver disease. Further ana-

lyses evaluating correlations between the ALBI score and the clinical course of disease in PBC and PSC patients are warranted.

2.33 FUT2 variant might modulate the course in secondary sclerosing cholangitis in critically ill patients (SC-CIP)

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Background and Aim: Variants in the FUT2 gene, which encodes a fucosyltransferase enzyme, have previously been associated with primary sclerosing cholangitis (rs601338) and plasma vitamin B12 levels (rs492602). Of note, these variants are in linkage disequilibrium and altered vitamin B12 levels seem to be associated with mortality in ICU patients (Sviri et al. Clin Nutr 2012). Secondary sclerosing cholangitis in critically ill patients (SC-CIP) is a progressive cholestatic disease with unknown pathophysiology with dismal prognosis. We hypothesized that FUT2 variants, which influenced plasma vitamin B12 levels in a genome-wide association study (GWAS) (Hazra et al. Nat Genet 2008), might modulate the course of SC-CIP. **Patients and Methods:** Having screened data of 4,641 cholangiography procedures performed in our department between January 2008 and April 2015, we identified 17 patients (age 33 – 80 years, 14 males; median bilirubin 3.5 mg/dl, gamma-GT 731 U/l, AP 428 U/L) with prior ICU treatment and signs of SSC. All patients were genotyped for the FUT2 variant rs492602 using a PCR-based assay with 5'-nuclease and fluorescence detection. Genotypes were analyzed with respect to data concerning ICU therapy and outcome, as well as liver stiffness quantified non-invasively by transient elastography. **Results:** The FUT2 variant was successfully genotyped in all patients and showed the following genotype frequencies: 8 patients (47.1%) were found to be wild-type (AA), 3 patients (17.6%) were heterozygous (AG), and 6 patients (35.3%) were homozygous mutation carriers (GG). During the follow-up period (median 467 days, range 39 – 3535 days), 2 patients required liver transplant and 3 patients died. In total, 8 patients developed liver cirrhosis and 8 presented with recurrent cholangitis. With respect to the FUT2 variant, 4 of the patients (44%) with the risk variant required liver transplant or died, compared to only one patient (13%) with FUT2 wild-type genotype. There was no obvious trend between genotypes and the development of cirrhosis or recurrent cholangitis. Both complications developed in 5 of the patients (55%) carrying the risk variant and 3 of the patients with wild-type genotypes (38%). **Conclusions:** Even though the investigated FUT2 variant does not seem to increase the risk of developing SC-CIP, its presence might be associated with a more severe disease course. Further studies including analysis of vitamin B12 homeostasis as well as other genetic variants affecting the progression of SC-CIP are mandatory.

2.34 Prevalence and Etiology of Elevated Aminotransferases in a Cohort of Patients referred to a German University Hospital

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Elevated liver enzyme values are associated with an increased risk of liver-specific mortality but are also a risk factor for non-hepatic diseases including diabetes mellitus type 2, metabolic syndrome, cardiovascular diseases and malignancies. Many liver diseases identified by an ALT screening can be treated successfully including prevention of development of clinical endpoints. Our aim was to study the prevalence and etiology of elevated liver enzymes in patients referred to a German University Hospital. **Methods:** 4,786 patients referred to the Department of Internal Medicine (General Internal Medicine, Gastroenterology, Cardiology, Nephrology), University of Kiel, were screened routinely for liver enzymes (ALT, AST, GGT, alkaline phosphatase, bilirubin) in a 6-month period. Patients with elevated aminotransferases but no evidence for viral associated hepatitis (Hepatitis A, B, C, D, E; CMV, EBV) were further analyzed in a retrospective cohort study. **Results:** Of the 4,786 patients 408 cases (8.5%) showed elevated AST/ALT values with no evidence for viral hepatitis. GGT values were elevated in 1,229 cases (25.6%) and alkaline phosphatase/bilirubin values in 175 cases (3.6%). By studying the individual health records 206 patients (50.5%) of the 408 cases with elevated ALT/AST values were analyzed for liver associated diseases. Non-alcoholic fatty liver disease (NAFLD) was diagnosed in 68 cases (33%) (NASH 47 cases, NASH associated cirrhosis 21 cases). Presumed alcoholic liver disease was found in 35 cases (16.9%) (alcoholic cirrhosis 16 cases). In 24 cases (11.6%) drug-induced liver toxicity and in 17 cases (8.2%) acute liver failure was recorded. Malignant liver tumors were found in 27 cases (13.1%) (Hepatocellular carcinoma 14 cases, metastases 13 cases). **Conclusion:** In our retrospective study patients referred to a tertiary hospital and screened for elevated AST/ALT values in half of the patients a liver-associated disease was diagnosed, most frequently caused by NAFLD, alcoholic liver disease or drug-induced liver toxicity. Thus abnormal liver enzymes should lead to focused investigation for underlying liver disease.

2.35 Prevalence of the sphingolipid storage diseases M. Gaucher and M. Niemann-Pick type B: results in a nation-wide screening project in 224 patients

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Background: M Gaucher (GD) and Niemann-Pick disease type B (NP-B) are monogenic autosomal-recessive storage diseases. They are caused by reduced activities of lysosomal β-glucocerebrosidase (GBA1 in GD) or acidic sphingomyelinase (ASM in NP-B). Gaucher leads to hepatosplenomegaly, osseous complications and a reduced life expectancy. The frequency of hepatocellular carcinoma is high in GD. Niemann-Pick disease type B leads to hepatosplenomegaly, pancytopenia and a prognostically poor emphysema, some of the patients develop liver cirrhosis. In Germany, between the actual number identified in these patients (respectively < 500) and the reference to the heterozygote frequency predicted number (> 2000) there is a discrepancy. Hepatosplenomegaly is almost never missing. For both there is an effective enzyme supplementation therapy, either recombinant glucocerebrosidase (imiglucerase, velaglucerase, taliglucerase) in GD or the recently approved recombinant sphingomyelinase (olipudase-α) for NP-B. **Methods:** Between 2008 – 2014 we carried out an activity determination of β-glucocerebrosidase in white blood cells (GBA) and chitotriosidase (CT) from EDTA blood in cooperation with clinical hematooncologists either from practice or hospital settings after exclusion of hepatic, malignant, purely hematological or infectious etiology of idiopathic hepatosplenomegaly. Gaucher-negative patients, CT-positive patients were offered determining the activity of ASM activity in leukocytes from EDTA blood to identify Niemann-Pick disease type B. **Results:** Five of the examined 224 patients had a pathological GBA1 activity, consistent with diagnosis of Gaucher disease. Two of a total of 15 patients with increased chitotriosidase activity, which were Gaucher-negative, exhibited a significantly decreased activity of ASM as a sign of Niemann-Pick type B. The age ranged from 0 – 88 years. Splenomegaly (≥ 5 multiples of normal, MN) was present in 85%, in 35% of patients studied it was severe (≥ 15 MN). Hepatomegaly (≥ 1.25 MN) was seen in 47%, only 7% being severely hepatomegalic (≥ 2.0 MN). In 57% thrombocytopenia was present, in 40% anemia was found as an expression of hypersplenism. Three of the seven sphingolipidoses had shown no typical storage cells (Gaucher or Niemann-Pick cells) in bone marrow smears. **Discussion:** The prevalence of Gaucher disease in patients with idiopathic splenomegaly is about 1:45. Patients with increased chitotriosidase activity and negative Gaucher screening should be examined on the sphingolipidosis Niemann-Pick disease type B. Gaucher treatment with enzyme replacement therapy and substrate reduction therapy has been established for decades and the recently introduced ERT at NP-B seems to be effective. In both diseases, there are abortive forms that can be clinically observed without specific therapy. The determination of CT activity is useful as a screening test and GD- and NP-B-negative cases should be analysed for other entities of lysosomal origin (e.g. cholesterol ester storage disease or Niemann-Pick type C).

Typical storage cells in KM may be missing, so a primary biochemical diagnosis is recommended in any idiopathic hepatosplenomegaly.

2.36 Comparative analysis of inflammatory biomarkers in spontaneous bacterial peritonitis and acute-on-chronic liver failure

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Purpose/Objectives: Novel biomarkers capturing portal hypertension, macrophage activation, and systemic inflammation have been suggested as diagnostic tools to identify patients with spontaneous bacterial peritonitis (SBP). Since acute deterioration of liver function, portal hypertension and systemic inflammation are frequent consequences of bacterial infection, we compared the accuracy of a panel of proposed biomarkers for SBP controlling for the severity organ failure in a matched case-control design. **Methods:** Forty cases of SBP were identified from prospective registries and were matched to 80 cirrhotic controls with bacterial infections other than SBP (n = 40) or with non-infectious systemic inflammatory response syndrome (SIRS) (n = 40) controlling for age, sex and Child-Pugh score using propensity score methods. Serum was analyzed for soluble CD 163, soluble urokinase-type plasminogen activator receptor (suPAR), mid-regional pro-adrenomedullin (MR-proADM), lipopolysaccharide-binding protein (LBP), intestinal fatty acid-binding protein (I-FABP), interleukin (IL)-6, IL-10, IL-1β and C-reactive protein (CRP). **Results:** Cases with SBP and matched controls without SBP did not significantly differ in baseline demographics, organ dysfunction scores (MELD, SOFA-CLIF) and systemic hemodynamic parameters. Hierarchical clustering of biomarker profiles revealed close correlations of serum concentrations of macrophage activation markers (sCD 163 with suPAR), cytokines (IL-6 with IL-10 and IL-1β), and acute phase proteins (LBP with CRP). In addition to the white blood cell count (WBC; AUROC 0.687 ± 0.053), only CRP and LBP levels identified patients with SBP, with a diagnostic accuracy of CRP for SBP higher than that of LBP (AUROC 0.781 ± 0.046 vs. 0.613 ± 0.054; p for difference = 0.003). MR-proADM, suPAR and sCD 163 were not increased in SBP cases compared to matched controls without SBP but strongly correlated with organ dysfunction scores including Child-Pugh, MELD and SOFA-CLIF (all p < 0.01). As a consequence suPAR, sCD 163 and MR-proADM were excellent indicators of established acute-on-chronic liver failure in the overall cohort (AUROCs 0.718 ± 0.049, 0.696 ± 0.051 and 0.699 ± 0.052, respectively) and predictors of 30-days survival (0.675 ± 0.060, 0.649 ± 0.062 and 0.716 ± 0.051) comparable to the composite MELD score (0.686 ± 0.061; p for difference > 0.5). **Conclusions:** Novel biomarkers of immune activation do not discriminate SBP as the cause of increased inflammation but identify patients with established organ failure and poor prognosis. In contrast, elevated levels of WBC, CRP and LBP indicate patients with established SBP.

2.37 Real Time Pressure Volume Loops in Cirrhosis: Characterization of Systolic and Diastolic Function and Validation of Doppler Indices with the Gold Standard

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Studies reporting abnormal left ventricular (LV) properties in cirrhosis have frequently used load-dependent indices. Aims: 1) to evaluate systolic and diastolic LV properties with the gold standard, LV conductance catheter, which allows obtention of real-time pressure volume loops, 2) to test the validity of Doppler-echocardiography indices compared to the gold standard, 3) to analyze the impact of severity of liver disease, neurohormonal activation and beta-blocker (BB) treatment on LV properties. **Methods:** Pressure-volume loops were obtained in 9 patients with cirrhosis (Child A:3;B:2;C:4; refractory ascites:2) in the context of liver transplant evaluation and 9 controls undergoing LV catheterization. Invasive gold-standard systolic (maximal elastance, Emax) and diastolic indexes (relaxation and stiffness) were correlated to Doppler-echocardiography indices including peak ejection intraventricular pressure difference (EIVPD) and strain rate (SR). Gold-standard validated Doppler indexes in cirrhosis (n = 59; Child A:15;B:25;C:19; refractory ascites:9) were compared to matched controls. The influence of the severity of liver disease, neurohormonal activation and BB was evaluated. Nonparametric tests were used. IRB approval was obtained. **Results:** Emax correlated only with EIPVD (r = 0.75, p < 0.01) and SR (r = 0.55, p = 0.02). No correlation was observed between Emax and ejection fraction (r =-0.18, p = 0.47), cardiac output (r = 0.3, p = 0.23) or dP/dtmax (r = 0.06, p = 0.81). Doppler indices of diastolic function (such as E/E') failed to correlate with reference indices of relaxation and stiffness. E/A correlated with end-diastolic pressure, which is associated to preload (R = 0.61 p = 0.009 for E/A). In 59 patients with cirrhosis, EIVPD was higher than in matched controls (p < 0.05), and was related to the severity of liver disease (Child class p = 0.01; MELD R = 0.45 p < 0.001), noradrenaline (R = 0.26, p = 0.05) and heart rate variability (SDNN, R =-0.43 p = 0.003). Patients with no ascites, diuretic responsive and refractory ascites had increasing EIPVD (p = 0.026) with no differences in SR (p = 0.848). E/A ratio was associated to Child class (p = 0.04). Patients on BB (n = 33) had lower peak EIVPD and SR (systolic), although higher than controls. Deceleration time (diastolic function) was significantly longer in patients on BB. **Conclusions:** Gold-standard indices of systolic function are increased in patients with cirrhosis. Systolic function as measured by validated Doppler indices in cirrhosis is increased compared to controls, even in refractory ascites. Noninvasive indices of diastolic function reflect LV filling pressures, but not gold-standard indices of diastolic function.

2.38 Regenerative potential of human pluripotent stem cell-derived MSCs in a Gunn rat liver injury model

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The Crigler-Najjar syndrome type 1 is characterized by massively increased levels of unconjugated bilirubin in the plasma because of the lacking of hepatic uridine 5'-diphospho-glucuronosyltransferase activity (due to a mutated UGT1A1 gene) which is crucial for bilirubin conjugation and excretion. The Gunn rat is an in vivo model for this disease. Current treatments have several limitations therefore alternative therapy strategies like (stem) cell transplantations are needed. Transplantation of primary hepatocytes is limited due to the dependence on donated organs. An alternative approach would be the increment of functional hepatocytes by cell transplantation. Studies have shown that co-transplanting hepatocytes with bone marrow-derived MSCs improves homing-in, engraftment and survival of the donor cells and that MSCs can transdifferentiate into hepatocytes. These properties make MSCs attractive tools in the treatment of acute liver injuries. However, the downside of procuring MSCs is that the number of cells that can be generated from a single human donor is limited due to their restricted long-term proliferation. To circumvent these drawbacks iMSCs (induced MSCs) were generated from (i) iPSCs derived from human fetal bone marrow MSCs and (ii) human embryonic stem cells- line H1. These were compared to human fetal bone marrow-derived MSCs. The iMSCs and the parental MSCs were transplanted into the spleen of partially hepatectomized Gunn rats (without immunosuppression). After a regeneration time of 1 week to 2 months the organs and sera were examined. Molecular, immunohistochemical and immunofluorescent-based analysis of rat liver tissue showed integration of human cells. Expression of human HNF4α as well as measureable human albumin levels in the sera provides evidences of trans-differentiation into hepatocytes. Further analysis revealed reduced levels of bilirubin. To surmize, human iMSCs are an alternative source for treating inherited liver diseases such as Crigler-Najjar syndrome type 1.

2.39 Severe drug-induced liver injury related to therapy with dimethyl fumarate

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Dimethyl fumarate (DMF) has been used for the treatment of severe psoriasis for many years and only recently been licensed as first-line therapy for relapsing-remitting multiple sclerosis (MS). Safety assessments have shown only moderate elevations of liver enzymes during DMF treatment. Here we report the case of a 26-year-old female with MS presenting with initial elevation of aminotransferases two weeks after initiation of DMF (Tecfidera©) treatment and progress to severe liver failure with jaundice despite prompt cessation of DMF therapy with the first appearance of elevated liver enzymes. The patient was on a dosage of 120 mg twice daily, which is only half of the recommended dosage for long-term treatment in MS patients. There was no co-medication or concomitant herbal intake, and other potential causes of acute liver failure were ruled out. The clinical and laboratory characteristics were well compatible with severe idiosyncratic drug-induced liver injury (iDILI) of a hepatitic type. Correspondingly liver histopathology showed severe hepatitis with hepatocellular bridging necrosis. The patient's condition improved spontaneously over several weeks. This is the first report of acute liver failure related to DMF treatment. In view of the increasing use of DMF, it is of specific relevance to acknowledge severe liver injury as potential complication.

2.40 Sicherheit und Effektivität eines getunnelten Verweilkatheters zur Aszitesdrainage bei Patienten mit Leberzirrhose und therapierefraktärem Aszites mit Kontraindikationen zur TIPS-Anlage

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Einleitung: Therapierefraktärer Aszites ist eine häufige Komplikation bei Patienten mit Leberzirrhose. Die Standardtherapie beinhaltet großvolumige Parazentesen, oder, vorzugsweise, die Implantation eines transjugulären portosystemischen Shunts (TIPS). Wiederholte großvolumige Parazentesen beeinträchtigen die Lebensqualität durch häufige Hospitalisierung. Die Implantation eines TIPS ist nicht bei allen Patienten möglich. Die Datenlage zu einer neuen Aszitespumpe ist noch spärlich und die Kosten hoch. In dieser Pilotstudie erfolgte die Evaluation der Sicherheit und Effektivität eines getunnelten peritonealen Verweilkatheters bei Patienten mit malignem oder therapierefraktärem benignem (zirrhotischem) Aszites und Kontraindikationen zum TIPS. **Patienten und Methoden:** 37 Patienten (17 mit benignem, 20 mit malignem Aszites) mit einer Parazentesehäufigkeit ≤ 2 Wochen, wurde zwischen 2012 und 2014 ein getunnelter Peritonealverweilkatheter implantiert. Die Analyse des klinischen und laborchemischen Verlaufes nach 30, 60 und 90 Tagen erfolgte retrospektiv. **Ergebnisse:** Von 37 Patienten entwickelten 8 (22%) Katheter-assoziierte Komplikationen die zur Explantation des Katheters führten (spontan-bakterielle Peritonitis; n = 4; Schmerzen um die Einstichstelle n = 3; Systemdefekt n = 1). 5 Patienten (14%) entwickelten ein akutes Nierenversagen nach Katheterimplantation und großvolumiger Drainage (2x transient, 2x permanent, 1x unbek.). Der mediane MELD Index 30, 60, und 90 Tage nach Katheterimplantation wurde durch die Intervention nicht signifikant beeinflusst. Bei 34 Patienten (92%) war innerhalb des Beobachtungszeitraums von 90 Tagen keine weitere Parazentese nötig. Das 30-, 60-, und 90-Tages-Überleben betrug 55, 45, und 40% bei Patienten mit malignem Aszites und 94, 88 und 82% in dem Kollektiv mit benignem (zirrhotischem) Aszites. **Schlussfolgerung:** Die Implantation eines getunnelten Peritonealverweilkatheters bei Patienten mit therapierefraktärem Aszites stellt eine vergleichsweise sichere und effiziente Therapieoption dar und könnte eine Reduktion der Hospitalisierungsrate in diesem Kollektiv bewirken.

2.41 iRhom2 regulates specific activation and trafficking of TACE and enhances the survival of TNF α mediated septic shock after LPS treatment

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Innate immune responses are vital for pathogen defense but can result in septic shock when excessive. TNFα is a powerful inflammatory cytokines, which controls infection and also acts as a key mediator of septic shock. TNFα which is synthesized as a membrane- bound precursor, is liberated from the cell surface by the TNFα converting enzyme (TACE, also known as ADAM17). We report that the inactive rhomboid family member iRhom2 interact with TACE and regulate TNFα shedding. Based on the results from an unbiased genetic screen, expression of either iRhom1 or iRhom2 lacking part of their extended N-terminal cytoplasmic domain plays important role in the specific activation of ADAM17 and confers resistance to TNF induced cell death via shedding of cell surface TNF receptor. In addition, iRhom2 was found to be critical for TACE maturation and trafficking to the cell surface. Gene-targeted iRhom2-deficient mice conformed that enhanced survial of TNFα induced septic shock after lipopolysaccharide (LPS) treatment. Our study has identified iRhom2 as a regulator of innate immunity that may be an important target for modulating sepsis and pathogen defense.

2.42 Spleen and liver stiffness measurement using transient elastography correlates well with hypertensive upper gastrointestinal bleeding risk – an evaluation of 143 patients

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Introduction: Portal hypertension is a common complication of chronic liver diseases and leads to development of esophageal and gastric varices. Variceal bleeding is associated with a high mortality rate. Measurement of hepatic venous pressure gradient (HVPG) and upper GI endoscopy are considered as gold standard for portal hypertension assessment. Because both methods are invasive and HPVG is available in tertiary centers only, non-invasive methods to assess severity of portal hypertension and consecutive risk of variceal bleeding are desirable. **Material and Methods:** We aimed at identifying the diagnostic performance of spleen- and liver stiffness measurement (SSM/LSM) using transient elastography (TE, Fibroscan®, Echosens) for non-invasive diagnosis of portal hypertension in detection of esophageal varices and prediction of bleeding episodes. We retrospectively correlated SSM/LSM with incidence of variceal bleeding in 143 patients who underwent spleen elastography between 2013 and 2015. **Results:** Of 113 included patients 19 (16.8%) had history of upper gastrointestinal variceal bleeding. The mean SSM of all patients was 42.9 kiloPascals (kPa), the mean LSM 22.0 kPa. Patients with prior episodes of bleeding had statistically significant higher SSM and LSM results (SSM 68.4 kPa, LSM 46.3 kPa) than those without history of bleeding (SSM 37.7 kPa, LSM 17.1 kPa, $p < 0.0001$). 75 patients (66.4%) underwent upper GI endoscopy simultaneous to SSM/LSM: 25 had no esophageal varices (EV) (average SSM 36.2 kPa, LSM 14.3 kPa), 16 showed EV grade 1 (average SSM 43.1 kPa, LSM 34.6), 21 EV grade 2 (average SSM 57.0 kPa, LSM 31.6 kPa) and 13 grade 3 (average SSM 65.3 kPa, LSM 35.9 kPa). Statistical analysis showed no significant differences between respective grades of EV. Using a calculated cut-off of 47.5 kPa for SSM and 26.0 kPa for LSM (Application of 95% confidence interval (CI)), SSM showed a sensitivity of 89% and specificity of 71% with a negative predictive value (NPV) of 0.92 (LSM sensitivity 84%, LSM specificity of 80%) regarding the risk of bleeding. When combining LSM (cut-off 26kPa) and SSM (cut-off 47.5kPa), a NPV of 1 (sensitivity 100%, specificity 56%) was found, **Conclusion:** We found that SSM and LSM using Fibroscan® as non-invasive approach for estimation of portal hypertension correlated well with a clinical history of upper gastrointestinal variceal bleeding (optimal SSM cut-off value: 47.5 kPa, optimal LSM cut-off value: 26.0 kPa), but not with the grade of EV. None of the patients with both SSM and LSM below cut-off experienced bleeding complications.

2.43 Tauroursodeoxycholsäure aktiviert das Ubiquitin-Proteasom System in Hepatitis B transgenen Zellen

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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 Ubiquitin-Proteasom System und molekulare Chaperone stellen eine intrazelluläre Qualitätskontrolle für die korrekte Faltung der Proteine dar. Ziel der vorliegenden Studie war die Untersuchung des chemischen Chaperons Tauroursodeoxycholsäure (TUDCA) auf das Ubiquitin-Proteasom System in HBs transgenen Zellen. **Methoden:** Eine stabil-HBs exprimierende Zelllinie (NIH 3T3) wurde etabliert und mit TUDCA und einem Proteasominhibitor behandelt. Zellkulturexperimente wurden mittels Immunhistochemie und Western Blot analysiert. **Ergebnisse:** Die Zugabe von TUDCA bewirkte in der HBs-transgenen Zelllinie eine erhöhte Ubiquitinierung und eine verminderte Akkumulation der durch Überexpression aggregierten HBV-Hüllproteine. Nach Zugabe des Proteasominhibitors wurde die durch TUDCA verminderte intrazelluläre Aggregation der HBV-Hüllproteine aufgehoben. TUDCA reduziert durch die Aktivierung des Ubiquitin-Proteasom Systems den Zellstress. **Schlussfolgerungen:** TUDCA zeigt einen Effekt auf das Ubiquitin-Proteasom System. Die hier identifizierten Eigenschaften von TUDCA könnten neue Therapieoptionen zur Behandlung von Protein-Speicherkrankheiten eröffnen. Die Ergebnisse unserer Studie tragen nicht nur zum besseren Verständnis der zellulären Pathophysiologie von HBV Oberflächenproteinen und Speicherkrankheiten („ER storage disease") bei, sondern zeigen ferner potentielle Wege zur Entwicklung neuer Therapieverfahren.

2.44 The distribution of surface antigen (HBsAg) components can distinguish between inactive carriers and active forms of Hepatitis B virus (HBV) infections

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Introduction: Different stages of HBV infections are associated with serum levels of HBV DNA and HBsAg, however, the distinction between active and inactive forms of HBV infections is difficult in many patients. The HBsAg itself consists of the components large (L-), middle (M-) and small (S-) HBsAg. The aim of our study was to investigate the composition of HBsAg during different stages of HBV infections. **Methods:** Total HBsAg and HBsAg components were quantified in serum samples of 150 patients (110 male, mean age 46.2 ± 15.4 (17 – 77) years) not receiving antiviral treatment including 41 inactive HBsAg-carriers (mean HBV DNA 3.1 ± 0.7 (range, 1.2 – 3.9 log10 copies/mL), 13 acutely infected patients, 41 with HBeAg negative and 55 patients with HBeAg positive chronic HBV infection (mean age 46.0 ± 15.6 (16 – 78) years) using an ELISA with well-defined monoclonal antibodies for L- and M- and commercial polyclonal antibodies for S-HBsAg/total HBsAg (HBsAg 6.0, Enzgnost, Siemens). **Results:** The mean levels of total HBsAg in patients with acute HBV infection were 3.5 ± 1.2 (range, 0.4 – 5.3), in patients with HBeAg negative and HBeAg positive HBV infection 3.6 vs. 4.1 log10 IU/mL ($p < 0.001$). In comparison to all active forms of HBV infections, inactive carriers had significantly lower mean total HBsAg levels (2.9 ± 1.2 (0.14 – 4.4) vs. 3.8 ± 0.8 (0.4 – 5.5) log10IU/mL; $p < 0.001$). The ratios of L-, M- and S-HBsAg in acutely infected patients were 9.9%, 9.3% and 80.8%, in HBeAg negative and positive patients 10.3% vs. 12.7% ($p = 0.024$), 6.5% vs. 5.6% ($p = 0.521$) and 83.8% vs. 81.7% ($p = 0.252$), respectively. In comparison to patients with active HBV infections, mean ratios of HBsAg components in inactive carriers were significantly lower for L- (4.2 ± 2.9 (0 – 11.3) vs. 11.5 ± 5 (1.9 – 25.5)%; $p < 0.001$, see Figure), higher for S- (92.8 ± 4.3 (80.4 – 100.0)% vs. 83.1 ± 7.4 (60.9 – 93.3); $p < 0.001$), and lower for M-HBsAg (3.0 ± 3.1(0 – 12.8) vs. 6.4 ± 5.2(0 – 35.5)%; $p < 0.001$). **Conclusion:** Patients with chronic inactive HBV infections show a distinct ratio of HBsAg components when compared to active forms of HBV infections. Quantification of HBsAg components may improve the identification of inactive carriers and individual risk stratification.

2.45 Genotypen-spezifische Prävalenz und Bedeutung natürlich vorkommender Precore-, Basal Core Promotor- und preS-Mutationen in einer großen europäischen Studienkohorte bei chronisch mit dem Hepatitis B Virus infizierten Patienten

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Einleitung/Ziele: In verschiedenen asiatischen Studien konnte gezeigt werden, dass Mutationen im basalen Core Promotor (BCP) und in der preS-Region des Hepatitis B Virus (HBV) mit der Entwicklung einer Leberfibrose/Zirrhose und/oder eines Hepatozellulären Karzinoms assoziiert sind. Bezüglich Mutationen im Precore-Gen (PC) ist die Datenlage eher kontrovers. Über die Relevanz dieser Mutationen in chronisch HBV-infizierten Patienten in der EU und den USA existieren jedoch nur wenige Daten. Um prognostische Marker auch in diesem Patientenkollektiv zu etablieren, wurde die genotypen-spezifische Prävalenz dieser Mutationen sowie die Bedeutung dieser für den Verlauf der Erkrankung in einer großen europäischen Studienkohorte von Patienten untersucht, die mit den HBV-Genotypen (GT) A bis E infiziert sind. **Methoden:** In der prospektiven, longitudinalen ALBATROS-Studie werden HBsAg-Träger, die keiner antiviralen Therapie bedürfen, über 10 Jahre untersucht. Baseline-Seren von 340 Patienten (GTA: 81, GTB: 28, GTC: 16, GTD: 192, GTE: 23) wurden analysiert. Das Fortschreiten der Infektion wurde bisher bis zu 5 Jahren Follow-Up (FU) kontrolliert. Die BCP/PC- und preS-Regionen wurden mittels nested PCR amplifiziert und mit populationsbasierter Sequenzierung analysiert. **Ergebnisse:** Die BCP Doppelmutation A1762T/G1764A konnte in unserer Kohorte mit einer Rate von 60% (203/340) detektiert werden. Die Prävalenz war bei GTE (91%) am höchsten, gefolgt von GTA, GTC und GTD (56 – 69%) und der niedrigsten Prävalenz bei GTB (29%). In 42% der Patienten (142/340) konnte die PC G1896A Mutation gefunden werden; mit hohen Prävalenzen in GTB, GTC und GTD (71, 56 und 54%) sowie niedrigen Prävalenzen bei GTA und GTE (10 und 9%). Außerdem wurde die PC G1896A/G1899A Doppelmutation mit einer mittleren Frequenz von 27, 18 und 13% in GTD-, GTB- und GTC-Patienten gefunden. In GTA-Patienten trat diese nur selten (3%) auf und konnte in GTE-Patienten nicht nachgewiesen werden (0%). Deletionen im preS-Bereich, die sich in ihrer Länge von 1 AS bis 41 AS unterschieden, traten in 8% der untersuchten Patienten mit der höchsten Prävalenz bei GTE (40%) und GTC (29%) auf. Bisher mussten 7 Patienten (GTA: 2; GTD: 4: GTE: 1) eine antivirale Therapie nach 1 – 4 Jahren FU beginnen. A1762T/G1764A wurde hierbei in 6/7 und G1896A/G1899A in 1/7 Patienten zu Baseline gefunden. **Zusammenfassung:** BCP und PC Mutationen konnten in unserer Studienkohorte mit großer Häufigkeit in einer HBV-Genotyp-spezifischen Verteilung nachgewiesen werden. Deletionen in der preS-Region traten hingegen nur mit einer niedrigeren Prävalenz hauptsächlich bei GTE und GTC auf. Interessanterweise hatten 6/7 HBsAg-Träger, die eine antivirale Therapie während des Studienzeitraums beginnen mussten, die BCP Doppelmutation zu Baseline, wobei PC Mutationen nur selten in diesen Patienten zu finden waren. Aufgrund dessen könnten BCP und PC Mutationen von großem klinischem Interesse als potentielle prognostische Marker auch bei europäischen HBsAg-Trägern sein.

2.46 Two week protein-enriched low-calorie diet shows rapid improvement of fatty liver as assessed by controlled attenuation parameter

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Introduction: Fatty liver (FL) is one of the most prevalent liver disorders increasing the risk of fibrosis and cirrhosis. FL occurs frequently in patients with diabetes. The aim herein was to assess for therapeutic effects of a short-term dietary intervention on FL, as quantified using the controlled attenuation parameter (CAP) during transient elastography. **Methods:** Sixty-six patients with FL received a 14-day low-calorie liver-specific diet containing 800 kcal/day (HEPAFAST: 41% protein, 29% carbohydrates, 24% fat, 6% fiber) and a maximum of 200 kcal/day through vegetable intake. The following parameters were assessed pre- and post-intervention: liver fat contents using the CAP algorithm; body composition; serum liver function tests and lipids. **Results:** All 66 patients (age 56 years [25 – 78], 52% women, BMI 31.7 kg/m2 [22.4 – 46.3]) successfully completed the study. A significant reduction in FL (14.3%; P < 0.001) was observed after only 2 weeks; median CAP score decreased from 296 dB/m (177 – 400) to 264 dB/m (100 – 353). Simultaneously, BMI decreased by 5%, 32% of the patients were reclassified into a lower BMI category, and body fat and visceral fat contents decreased by 7%. Serum triglyceride, LDL and GGT also decreased (all P < 0.001). Interestingly, 11 patients (73% women) demonstrated a CAP increase despite improvements in body composition, thus were classified as hepatic non-responders. A subgroup analysis of the responders revealed a decrease of 17% in median CAP scores from 311 to 263 dB/m. When comparing diabetics with non-diabetics (24% vs. 76%), equal improvements of liver fat, body composition, serum liver function tests and lipid profiles were observed (all P > 0.05). **Discussion:** This non-invasive elastography-based study demonstrates for the first time improvements in liver fat, as quantified by CAP, after a short-term protein-enriched low-calorie diet. The dietary intervention reduced body weight and improved body and liver composition in diabetics and non-diabetics alike.

2.47 Untersuchung des Langzeitverlaufs von Patienten mit einer niedrig-replikativen chronischen Hepatitis B-Infektion, die keine antivirale Therapie erhalten: 2 Jahres-Daten einer deutschen prospektiven Studie (ALBATROS Studie)

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Einleitung: Studien aus Asien zeigten, dass die HBsAg-Konzentration sowie die Ausgangsviruslast prognostisch bedeutsam sind für einen spontanen HBsAg Verlust bei niedrig-replikativer HBV-Infektion. Ziel der folgenden Studie war es, die Dynamik von viralen und biochemischen Parametern zu analysieren, welche mit einem HBsAg Verlust bzw. einer HBsAg Serokonversion bei inaktiven HBsAg Trägern assoziiert sind. **Material und Methoden:** 672 Patienten mit HBeAg-negativer HBV-Infektion wurden prospektiv untersucht. Leitlinienkonform bestand bei keinem Patienten die Indikation zur antiviralen Therapie bei Studieneinschluss. Biochemische und virologische Parameter sowie nicht-invasive Fibrosemarker wurden zu Baseline sowie 1x/Jahr während des Follow-up erfasst und zwischen den folgenden Gruppen verglichen: Patienten mit persistierendem positivem HBsAg (Gruppe A), Patienten mit HBsAg Verlust (Gruppe B) und Patienten mit HBsAg Serokonversion (Gruppe C) während der ersten beiden Follow-up – Jahre. **Ergebnisse:** Verlaufsdaten nach 1, 2, 3, 4 und 5 Jahren liegen aktuell von 441, 207, 143, 75 und 27 Patienten vor. 12 Patienten haben ihr HBsAg innerhalb von 2 Jahren verloren, und 8 von 12 erzielten eine HBsAg Serokonversion. Patienten ohne HBsAg Verlust (Gruppe A) hatten höhere HBsAg Titer zu Baseline als Patienten mit HBsAg Verlust (Gruppe B) (p < 0,001) und HBsAg Serokonversion (Gruppe C) (p < 0,0001). In Gruppe B+C betrug der logHBsAg Abfall 2,33 bzw. 3,76 pro Jahr im Vergleich zu -0,05 bei den verbliebenen Patienten (n = 195) ohne HBsAg Verlust (p < 0,0001). Es zeigte sich kein signifikanter Unterschied hinsichtlich des HBsAg Abfalls zwischen Gruppe B+C. Die Höhe der HBV DNA zu Baseline war höher in Gruppe A als in Gruppe B und C (p = 0,021 A vs. B; p = 0,001 A vs. C) ohne signifikanten Unterschied der jährlichen Virusdynamik. AST war signifikant höher in Gruppe A vs. C (p = 0,016). HDL war höher in Gruppe A als in Gruppe B und am niedrigsten in Gruppe C (p = 0,046). ALT, GGT, LDL und Gesamtcholesterin zeigten keine signifikanten Unterschiede innerhalb der 3 Patientengruppen. **Schlussfolgerung:** Nach 2 Jahren prospektivem Follow-up von 672 Patienten mit niedrig-replikativer chronischer HBV-Infektion waren HBsAg Verlust und Serokonversionsraten niedrig (6% bzw 4%). Neben der absoluten HBsAg Konzentration zu Baseline war der dynamische HBsAg Abfall ein wesentlicher Parameter zur Vorhersage von HBsAg Verlust und Serokonversion.

2.48 γ-GT is associated with splanchnic thrombotic events, CRP predicts survival in patients with myeloproliferative neoplasms

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Background: Myeloproliferative neoplasms (MPN) are associated with an increased risk of thrombotic events, especially in polycythaemia vera (PV) and essential thrombocythaemia (ET). Thrombosis of splanchnic vessels (SVT) may lead to portal hypertension and varices with increased bleeding risk rendering anticoagulation difficult. The identification of risk factors for SVT development might improve the management of MPN patients. In this study, we aimed to identify risk factors for SVT in MPN patients. **Methods:** The retrospectively analysed training cohort included 86 MPN patients which were referred to our hospital between 2009 and 2014, and 40 SVT patients without MPN. 86 patients suffered from MPN (PV n = 18, ET n = 16, MF n = 40, undetermined n = 12) and 40 patients with SVT but without MPN. Clinical and laboratory data were collected and statistically analysed. The prognostic relevance of identified risk factors was validated using a validation cohort of 181 patients from the MPN registry of the Study Alliance of Leukemia. **Results:** In the training cohort, 33 patients (38.4%) with MPN had SVT compared to 76 patients (40.2%) in the validation cohort. Patients with SVT had higher AST, ALT and γ-GT values (p < 0.05). There was no difference between AST, ALT and γ-GT in SVT patients with and without MPN. We further evaluated the MPN patients of the training and validation cohort regarding risk factors for survival and SVT development. In univariate analysis, γ-GT, CRP and bilirubin were associated with the presence of splanchnic vein thrombosis (p < 0.05). In multivariate analysis, γ-GT was a risk factor for the presence of SVT in MPN patients (p < 0.05). LDH, γ-GT and CRP were associated with patient survival in univariate analysis. CRP was also associated with patient survival in multivariate analysis. **Conclusions:** Elevated γ-GT in patients with MPN might be an indicator for splanchnic vein thrombosis and should therefore lead to further diagnostic examination in these patients.

3. Metabolism and Transport

3.1 Die lithogene Variante D19H im Cholsterintransporter Abcg8 führt zu verminderter Phospholipidsekretion in Mäusen

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Hintergrund: Die Gallensteinerkrankung als „Volksleiden" betrifft immer mehr Menschen, obgleich verschiedene Ursachen zugrunde liegen. Meist kommt es zu einer Übersättigung der Galle mit Cholesterin, wobei dieses ausfällt und die Steine verursacht. Cholesterin wird mittels des heterodimeren Transporters ABCG5/G8 aus dem Hepatozyten in die Galle transportiert. Etwa 25% aller Gallensteine sind genetisch prädisponiert, wobei die Variante D19H im ABCG8-Gen als Faktor identifiziert werden konnte (Grünhage et al., 2007). Um diese Variante und die damit eventuell verbunden Änderungen in der Zusammensetzung der Gallenflüssigkeit zu untersuchen, wurden Mäuse generiert, die die Variante tragen. **Methodik:** Die Varianten D19H im Abcg8-Gen und R51C im Abcg5-Gen, welche im Kopplungsgleichgewicht liegen, wurden mittels BAC-Technologie, ES-Zell-Elektroporation und anschließender Blastozysteninjektion in Mäuse eingebracht. Neben Expressionsanalysen wurden die Tiere einer Galle-Fistel unterzogen, die Galle zu verschiedenen Zeitpunkten gesammelt, die Lipidzusammensetzung gemessen und mit Kontrollen verglichen. **Ergebnisse:** Die Tiere entwickelten sich normal, waren fertil und zeigten keinerlei Auffälligkeiten. Die Expression von Abcg5 und Abcg8 war weder in der Leber noch in Duodenum, Jejunum und Ileum signifikant verändert. Interessanterweise zeigte sich eine Erhöhung der Expression des alternativen Cholesterintransporters Abcg1 in den Variantenträgern. Dort war außerdem das Cholesterin-veresternde Enzym Acat2 erniedrigt. Bezogen auf die Gallensalzkonzentration zeigten die männlichen Variantenträger erhöhten Gallefluss und erhöhte Cholesterinsekretion. Phospolipide waren dagegen leicht vermindert. **Schlussfolgerungen:** Die Variante D19H im Abcg8-Gen führt in Mäusen zu einer erhöhten Cholesterinsekretion bei vermindertem Phospholipid-Output. Dies könnte eine Erklärung für die genetische Prädisposition bei Cholelithiasis sein.

3.2 Deficiency of the oncostatin M receptor affects the pathogenesis of non-alcoholic fatty liver disease in a context dependent manner

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Background: The early phase of inflammation is characterized by the release of cytokines/chemokines from activated neutrophils, monocytes and dendritic cells. One of the most strongly secreted cytokines is the interleukin-6-type cytokine oncostatin M (OSM). Since OSM itself is a strong inducer of chemokines, particularly in tissue-resident cell types like fibroblasts, it is considered to promote the inflammatory response. Surprisingly, it has recently been shown to execute protective functions in high-fat diet-induced obesity and related metabolic disorders in mice. Its role in the development of non-alcoholic fatty liver disease (NAFLD) and in conjunction with hypercholesterolemia, however, has not been addressed so far. **Methods:** C57Bl/6, Osmr-/-, Ldlr-/- single knockout and Ldlr-/-, Osmr-/- double knockout mice were fed a high-fat diet for 12 weeks. Weight gain was documented on a weekly basis. After 12 weeks mice were sacrificed. Liver weight was evaluated; steatosis and cellular composition were analyzed by immunohistochemistry. Serum levels of cholesterol and leptin were determined by HPLC and ELISA, respectively. Expression of liver enzymes was monitored by qPCR analyses. **Results:** Confirming the published results, Osmr-/- mice gained significantly more body and liver weight in contrast to C57Bl/6 mice. Serum cholesterol levels were increased more than two-fold in knockout compared to wild-type animals. However, on an Ldlr-/- background displaying hypercholesterolemia the phenotype was reversed and Ldlr-/-Osmr-/- double knockout mice gained less body and liver weight, showed lower blood glucose levels as well as serum cholesterol and leptin levels. Preliminary analyses of liver enzymes indicated a reduction in the expression of enzymes involved in fatty acid synthesis (Fasn, Elovl6, Acc, Scd1), no differences in long-chain acyl-coenzyme A synthetases (Acsl), but altered cholesterol metabolism in Ldlr-/- Osmr-/- double knockout mice. **Conclusion:** OSM exerts protective as well as pathogenic influences on the establishment of fatty liver disease depending on the genetic background and metabolic status. Further in depth characterization of the different mouse models will help to dissect the molecular mechanisms underlying this ambivalence. Thereby, this study will help to clarify if OSM might indeed serve as a therapeutic agent for the treatment of obesity and related metabolic disorders as recently suggested.

3.3 Development of a new modified western diet to induce NASH with obesity and insulin resistance in mice

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Background & aims: The alarming increase of obesity becomes a major global health issue. Obesity is often associated with insulin resistance, type II diabetes and non-alcoholic fatty liver disease (NAFLD) and may result in the metabolic syndrome. There are many contributing factors to obesity, but diet is one of the most relevant. Feeding studies with rodents like mice were often used to investigate the mechanisms leading to obesity and its associated co-morbidities, but the applied diets often did not induce the same phenotype like in human metabolic syndrome. High fat diets with saturated fatty acids induce obesity, insulin resistance and steatosis in mice, however mice do not develop hepatic steatosis with inflammation (NASH). Methionine- and choline-deficient diets as well as diets high in cholesterol and cholate induce NASH, but mice lose weight and are not insulin-resistant. Here we designed a new modified western diet that caused obesity, insulin resistance and NASH in mice. **Methods:** Male C57BL/6 mice were fed chow a high fat diet (HFD) (25 g/100 g lard) or a modified western diet (mWD) containing high fat (25 g/100 g soybean oil) and cholesterol for 20 weeks. **Results:** Mice fed a mWD significantly gained weight and increased their body fat mass 2.5-fold compared to chow fed mice. In an oral glucose intolerance test mice fed a mWD or a HFD were glucose intolerant with slightly increased insulin levels. In comparison to chow fed animals, serum parameters for liver inflammation like ASAT, ALAT and cholinesterase were elevated after feeding a mWD but after feeding HFD. Histological scoring of the liver revealed steatohepatitis (NASH) in mWD-fed mice and only steatosis without inflammation in HFD-fed mice. Gene expression analysis detected an up-regulation of chemokines (CCL2), pro-inflammatory cytokines (IL-1β, TNFα) and immune cell infiltration (CD68) in livers of mWD-fed, but not HFD-fed mice. Still, both mWD and HFD feeding was accompanied by enhanced expression of markers of hepatic insulin resistance (PTP1B, FGF21). **Conclusion:** In contrast to mice, which receive a high fat diet based on saturated fatty acids, mice fed a modified western diet with high amounts of unsaturated fatty acids and cholesterol developed obesity, insulin resistance and hepatic inflammation. Mice fed a mWD therefore are a potential better model for human metabolic syndrome and NASH than mice fed a HFD.

3.4 Ammoniak induziert NADPH-Oxidase 4-vermittelt oxidativen Stress in kultivierten Rattenastrozyten

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Einleitung: Die hepatische Enzephalopathie (HE) ist ein im Gefolge akuter und chronischer Leberfunktionsstörungen auftretendes neuropsychiatrisches Syndrom. Sie ist die Folge einer zerebralen Hydratationsstörung (1) die vergesellschaftet ist mit einer erhöhten Expression von Biomarkern für oxidativen/nitrosativen Stress (2) und Seneszenz (3). In der vorliegenden Untersuchung wurde die Bedeutung der NADPH-Oxidase (NOX) 4 für durch Ammoniak in kultivierten Rattenastrozyten induzierten oxidativen/nitrosativen Stress und Seneszenz überprüft. **Material und Methoden:** mRNA Expressionslevel von NOX4 und Growth Arrest and DNA Damage (GADD45) α wurden mittels Realtime-PCR quantifiziert. Die NOX4-Proteinexpression und oxidierte DNA/RNA (8-oxo-(deoxy)-guanosine/8OH(d)G) wurden durch Immunfluoreszenzanalyse nachgewiesen. Die Proliferation wurde durch fluorimetrische Quantifizierung der Hoechst34580 Fluoreszenz gemessen. **Ergebnisse:** Unter Kontrollbedingungen wurde nur eine schwache anti-NOX4 Immunoreaktivität in kultivierten Rattenastrozyten beobachtet. Demgegenüber steigerte NH4Cl (5 mmol/l) nach 24, 48 und 72 h die anti-NOX4 Immu-

noreaktivität insbesondere im Bereich des Zellkerns deutlich. Gleichzeitig wurde ein zeitabhängiger, signifikanter Abfall der NOX4 mRNA-Spiegel 24, 48 und 72 h nach NH4Cl (5 mmol/l)-Exposition beobachtet. Gegenüber unbehandelten Kontrollen induzierte NH4Cl (5 mmol/l 24, 48 und 72 h) eine deutliche Steigerung der anti-8OH(d)G Immunoreaktivität in kultivierten Astrozyten. Sowohl die durch NH4Cl (5 mmol/l 24, 48 und 72 h)-gesteigerte anti-NOX4-, wie auch die anti-8OH(d)G-Immunoreaktivität waren hemmbar durch den Glutaminsynthetasehemmstoff Methionin-Sulfoximin (MSO). Durch Transfektion mit NOX4-spezifischer siRNA (80nmol/l, 72 h) konnten die NOX4 mRNA Spiegel in kultivierten Rattenastrozyten um ca. 60% verringert werden und gleichzeitig die durch NH4Cl (5 mmol/l, 72 h)-induzierte Steigerung der anti-NOX4- und anti-8OH(d)G-Immunoreaktivität deutlich vermindert werden. Die Transfektion kultivierter Rattenastrozyten mit NOX4-spezifischer siRNA hemmte die durch NH4Cl (5 mmol/l, 72 h)-induzierte mRNA-Expressionssteigerung des zellzyklus-inhibitorischen Gens GADD 45α und den damit assoziierten Proliferationsrückgang kultivierter Astrozyten. **Diskussion:** Die Daten der vorliegenden Untersuchungen weisen auf eine wichtige Rolle der NOX4 für durch Ammoniak in kultivierten Rattenastrozyten induzierten oxidativen Stress und Astrozytenseneszenz. **Literatur:** [1] Häussinger D, Laubenberger J, vom Dahl S, Ernst T, Bayer S, Langer M, Gerok W, Hennig J. Proton magnetic resonance spectroscopy studies on human brain myo-inositol in hypo-osmolarity and hepatic encephalopathy. Gastroenterology. 1994; 107:1475 – 80. [2] Görg B, Qvartskhava N, Bidmon HJ, Palomero-Gallagher N, Kircheis G, Zilles K, Häussinger D. Oxidative stress markers in the brain of patients with cirrhosis and hepatic encephalopathy. Hepatology. 2010; 52:256 – 65. [3] Görg B, Karababa A, Shafigullina A, Bidmon HJ, Häussinger D. Ammonia-induced senescence in cultured rat astrocytes and in human cerebral cortex in hepatic encephalopathy. Glia. 2015; 63:37 – 50.

3.5 Ammoniak hemmt die LPS-induzierte Mikrogliaaktivierung und Transkription pro-inflammatorischer Zytokine in Astrozyten/Mikroglia Kokulturen

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Einleitung: Die hepatische Enzephalopathie (HE) ist ein neuropsychiatrisches Syndrom und eine wichtige Komplikation bei akuten und chronischen Lebererkrankungen. Neuere Untersuchungen zeigen, dass Ammoniak, ein Haupttoxin der HE, Mikroglia zwar aktiviert, aber nicht die Transkription pro-inflammatorischer Gene wie z. B. inflammatorische Zytokine aktiviert (1). In der vorliegenden Studie wurde die Wirkung von Ammoniak, einem Haupttoxin der HE, auf die durch Lipopolysaccharid (LPS)-induzierbare Transkriptionssteigerung von Mikrogliaaktivierungsmarkern und Zytokinen sowie deren Sekretion überprüft. **Material und Methoden:** Die mRNA Expression von Mikrogliaaktivierungsmarkern und pro-inflammatorischen Zytokinen wurden durch Realtime-PCR in mRNA-Präparationen kultivierter Mikroglia und Astrozyten und Zytokinkonzentrationen wurden im Zellkulturüberstand mit Luminex® Multiplex Zytokin Assays gemessen. **Ergebnisse:** Die Behandlung kultivierter Mikroglia mit LPS (100 ng/ml, 18 h) steigerte die Transkription des Mikrogliaaktivierungsmarkers CD 14 und der Zytokine IL 1α, IL 1β, IL-6 und TNFα. In Mikrogliamonokulturen beeinflusste die gleichzeitige Behandlung mit NH4Cl (5 mmol/l) die LPS-induzierte Transkriptionssteigerung von CD 14, IL 1α, IL 1β und IL6 nicht und verstärkte die TNFα mRNA-Spiegel signifikant gegenüber nur mit LPS-behandelten MIkroglia. Demgegenüber wurde In Astrozyten/Mikroglia-Kokulturen die durch LPS induzierten mRNA-Expressionssteigerungen von CD 14, IL 1α, IL 1β, TNFα und IL 6, nicht aber des anti-inflammatorisch wirksamen IL 10 signifikant durch NH4Cl (5 mmol/l) gehemmt. Die LPS-induzierte Sekretion der Zytokine MCP1, TNFα, IL 1α, IL 1β, IL 6 und IL 10 differierte zwischen Astrozyten-, Mikroglia- und Astrozyten/Mikroglia-Kokulturen quantitativ und qualitativ. Während in LPS-behandelten Astrozyten überwiegend MCP1 und IL-6 im Zellkulturüberstand nachgewiesen werden konnten, fanden sich im Überstand LPS-behandelter Mikroglia zusätzlich die pro-inflammatorischen Zytokine TNFα, IL 1αα und IL 1β. Die gleichzeitige Behandlung kultivierter Mikroglia mit NH4Cl (5 mmol/l) verminderte die LPS-induzierten Konzentrationserhöhungen von MCP1 und der potentiell anti-inflammatorisch wirksamen Zytokine IL 6 und IL 10 im Überstand kultivierter Mikroglia. Demgegenüber wurde keine verminderte LPS vermittelte Freisetzung von MCP1, TNFα, IL 1α, IL 1β, IL 6 und IL 10 in Gegenwart von NH4Cl (5 mmol/l) in mit Astrozyten kokultivierten Mikroglia gefunden. **Diskussion:** Ammoniak hemmt die durch LPS-induzierte Transkription pro-inflammatorischer Zytokine in Astrozyten/Mikroglia-Kokulturen aber nicht in monokultivierten Mikroglia. Die anti-inflammatorische Wirkung von Ammoniak auf Mikroglia könnte im Zusammenhang stehen mit der an post mortem Hirnproben von Zirrhosepatienten mit HE gemachten Entdeckung, dass Mikroglia bei HE aktiviert, aber nicht reaktiv ist und keine erhöhten zerebralen mRNA-Spiegel pro-inflammatorischer Zytokine gefunden werden (1). **Literatur:** [1] Zemtsova I, Görg B, Keitel V, Bidmon HJ, Schrör K, Häussinger D. Microglia activation in hepatic encephalopathy in rats and humans. Hepatology 2011; 54:204 – 215

3.6 Ammoniak induzierte miRNA Expressionsänderungen in kultivierten Rattenastrozyten

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Einleitung: Die hepatische Enzephalopathie (HE) ist ein neuropsychiatrisches Syndrom, welches mit der zerebralen Bildung von osmotischem und oxidativ/nitrosativem Stress assoziiert ist. Ammoniak, ein Schlüsseltoxin in der Pathogenese der HE, induziert über Bildung von oxidativen Stress Astrozytenseneszenz. Die hierbei zugrundeliegenden molekularen Mechanismen sind nur wenig verstanden. MicroRNAs (miRNAs) sind kurze, nicht-kodierende, einzelsträngige RNA Moleküle, die die Genexpression auf posttranskriptioneller Ebene regulieren. Da microRNAs (miRNAs) die Expression zellzyklussteuernder Gene regulieren können und ihre Expression durch oxidativen Stress moduliert werden kann, wurde in der vorliegende Studie untersucht, ob Astrozytenseneszenz durch Ammoniak-induzierte miRNA Expressionsänderungen vermittelt wird. **Methoden:** Die miRNA-Expressionsanalyse in unbehandelten oder mit Ammoniak (5 mmol/l NH4Cl, 48 h)-behandelten kultivierten Rattenastrozyten wurde mittels Agilent Rat miRNA Microarray durchgeführt und durch quantitative miRNA-Realtime-PCR (miQPCR) validiert. Für die Transkriptomanalyse wurde der Affymetrix Rat Genome Microarray verwendet und die gefundenen Genexpressionsänderungen wurden durch Realtime-PCR validiert. Potentielle Zielgene durch Ammoniak verringert exprimierter miRNA-Spezies wurden mithilfe bioinformatischer Tools (miRWalk) ermittelt. Die Validierung bioinformatisch vorhergesagter potentieller Zielgene erfolgte durch Transfektion kultivierter Astrozyten mit miRNA-Inhibitoren. Die Astrozytenproliferation wurde durch fluorimetrische Quantifizierung der Hoechst34580-Fluoreszenz bestimmt. Die Expression der HO-1 wurde auf Protein- und mRNA-Ebene mittels Western-Blot bzw. Realtime-PCR gemessen. **Ergebnisse:** An kultivierten Astrozyten wurden mittels Microarray-basierter miRnom- und Transkriptomanalyse 43 herunterregulierte miRNA-Spezies und 142 hochregulierte Gene in NH4Cl (5 mmol/l, 48 Std.) behandelten Astrozyten identifiziert. Die durch Microarray-Analyse identifizierten miRNA- und Genexpressionsänderungen wurden nachfolgend exemplarisch durch quantitative Realtime-PCR validiert. Mithilfe bioinformatischer Analysetools wurden 43 potentielle Zielgene von durch Ammoniak-vermindert exprimierten miRNA-Spezies unter den durch Ammoniak hochregulierten Genen identifiziert. Weitere Untersuchungen fokussierten auf die Bedeutung von durch Ammoniak herunterregulierter miRNA-Spezies für die Expressionssteigerung der Hämoxygenase-1 (HO-1). Eine Inhibition von HO-1 regulierenden und durch Ammoniak herunterregulierten miRNA-Spezies steigerte die HO-1 mRNA- und Proteinexpression und hemmte die Astrozytenproliferation in HO-1-abhängiger Weise. Sowohl die Hemmung der ammoniakinduzierten Hochregulation der HO-1 durch Taurin (5 mmol/l) als auch der HO-1 Hemmstoff Zinn-Protoporphyrin IX verhinderten die ammoniakinduzierte Proliferationshemmung und Seneszenz. **Diskussion:** Die Befunde der vorliegenden Studie lassen vermuten, dass Ammoniak-induzierte Astrozytenseneszenz über Expressionserniedrigungen spezifischer potentiell die HO-1 Expression regulierender miRNA-Spezies vermittelt wird, in deren Folge die HO-1 hochreguliert wird.

3.7 Antiapoptotic and antioxidative protein ALR in Cholestatic Liver Diseases – Do bile acids regulate ALR expression via Egr1?

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Cholestatic liver diseases result when the excretion of bile acids from the liver is interrupted. It has been shown that inflammation contributes to liver injury during cholestasis and previous studies indicated that bile acids up- regulate early growth response fator-1 (Egr1) through the activation of MAPK signaling pathway. Egr1 is responsible for the development of the inflammation through up-regulation of several pro-inflammatory genes. Augmenter of liver regeneration (ALR) is a hepatotrophic factor with anti- oxidative and anti-apoptotic properties which has been found to be highly expressed in regenerating livers after tissue loss or insults through toxic substances. Nevertheless, little is known about the impact of ALR in cholestasis. Therefore, the aim of our study is to investigate the potential role of bile acids in regulating ALR expression as a protective protein in cholestasis, and to determine which transcription factors might regulate the tissue-specific expression of ALR. Promoter studies of ALR gene (-733/+527) revealed several potential regulatory elements of Egr1. Luciferase assay was performed using two different constructs of ALR promoter cloned into pGL2 vector (-733/+240, -733/+527). The assay revealed that co-transfection with Egr1 significantly induced the activity of only one of the two ALR promoter constructs (-733/+527) in both HepG2 and Huh7 cells. In addition, co-transfection with a dominant negative EGR1 (dnEGR1) expression plasmid revealed no activity of either promoter constructs. Furthermore, different bile acids (known to be associated with cholestasis) were used to analyze their potential for ALR promoter activation. To further verify the results of the luciferase assay, an in-vitro model was used to imitate cholestasis. Expression of ALR in HepG2 and Huh7 cells after bile acid treatment was analyzed on mRNA and protein levels using qPCR and western blot techniques respectively. Additionally we investigate the role of Egr1 in regulating ALR expression by preforming EMSA analysis.

3.8 Arginase 1 deficiency: long-term follow-up of the original patients

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Arginase 1, the enzyme responsible for the last step of the urea cycle, catalyzes the hydolysis of arginine to urea and ornithine. In contrast to other urea cycle defects, its deficiency usually does not cause early and catastrophic hyperammonemia. Instead, this extremely rare disease typically presents with progressive cerebral and motoneuron disorder (spastic paraplegia and seizures) and/or a hepatic phenotype with neonatal cholestasis, acute liver failure, or liver fibrosis, making it a prime example of how pathology in the liver affects other organs. The underlying pathogenetic mechanisms have not been fully understood yet. However, the accumulation of arginine and consecutive abnormalities in the metabolism of guanidino compounds and nitric oxide are thought to be involved in pathophysiological processes in the CNS. In addition, induction of arginase 2 enzyme function may be involved in the pathogenesis of liver disease in arginase 1 deficiency. Treatment of arginase 1 deficiency resembles that of other UCDs and consists of a strict restriction of protein intake to reduce arginine levels and of nitrogen scavenging drugs to prevent hyperammonemia but often cannot prevent disease progression. We provide clinical data on the long-term follow-up of the first patients ever described with this extremely rare disorder of the urea cycle.

3.9 Characterization of recombinant human monoclonal antibodies against the Bile Salt Export Pump

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Background: Inhibitory, Bile Salt Export Pump (BSEP)-reactive IgG-class antibodies cause phenotypic recurrence of BSEP deficiency in some patients after liver transplantation for Progressive Familial Intrahepatic Cholestasis Type 2 (PFIC-2). A detailed characterization of these disease-causing antibodies is key to elucidate the molecular mechanism of this emerging disease. **Methods:** BSEP-reactive B cells were isolated from peripheral blood of a patient with a high anti-BSEP titer by fluorescence-activated single cell sorting. Corresponding full-length immunoglobulin heavy and light chain variable region gene transcripts were amplified by single cell RT-PCR and cloned into eukaryotic expression vectors to enable the in vitro production of the respective monoclonal antibodies. These monoclonal antibodies were characterized by immunofluorescence studies of rat liver slices and transiently transfected cells, Western blot, FACS analysis, in situ rat liver perfusion and in vitro BSEP transport assays. **Results:** Immunofluorescent staining of fixed, transiently transfected human embryonal kidney (HEK293) cells identified several BSEP-reactive antibodies that also stained canalicular patterns on fixed rat liver slices. In contrast, only a few recognized denatured rat BSEP on Western blots, while all antibodies detected its denatured human homologue. FACS analysis of antibody-treated, unpermeabilized cells transiently expressing BSEP revealed BSEP-specific staining that was absent on empty control cells. BSEP-reactive antibodies reached the canalicular space of in situ perfused rat livers within two hours. Finally, in vitro vesicular transport assays showed clone-dependent influence on transport activity. **Conclusion:** We provide characterization of a novel set of patient-derived, monoclonal antibodies directed against extracellular features of BSEP. These include species that are BSEP-inhibitory, -neutral, and, strikingly, -stimulatory in vitro. The antibodies and their monovalent Fab derivatives may have great potential in applied research as well as in future clinical applications.

3.10 Deficiency of calcium-independent phospholipase A2 beta with aging causes biliary epithelial ductular reaction associated with increased bile acids in enterohepatic circulation

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Background/Aims: Calcium-independent phospholipase A2 beta (iPLA2-beta) generates lysophosphatidylcholine which is recognized as a macrophage 'fine me' signal for removal of apoptotic cells. We have shown that iPLA2beta deficiency causes an accumulation of apoptotic hepatocytes associated with a decrease of serum and hepatic lipids including cholesterol. We here determined whether iPLA2beta deficiency in aged mice can result in abnormalities in the hepatobiliary system and the alterations of bile acid contents. **Methods:** Control (WT) and whole body male iPLA2beta-/- mice at 19 – 22 months old were used. Bile acid profiles were measured by liquid-chromatography mass spectrometry. Functional assays included qRT-PCR, histology, and immunohistochemistry (IHC). **Results:** Livers of aged iPLA2beta-/- mice exhibited increased IHC staining of α-smooth muscle actin and biliary epithelial marker cytokeratin 19 compared with those of aged WT mice. This was concomitant with increases of total, hydrophilic, hydrophobic and secondary bile acids in the bile. The increases of hydrophobic secondary bile acids such as deoxycholic and chenodeoxycholic acids were observed in liver and intestine of iPLA2beta-/- mice, which was associated with increased mRNA expression of CYP7A1 and CYP8B1, and decreased expression of bile acid export genes BSEP and MRP3 in liver as well as OSTα and OSTβ in intestine. In the feces of mutant mice, we observed an increase of deoxycholic acid (suggesting increased dehydroxylation by luminal bacteria), but decreases of tauro-ursodeoxycholic acid as well as tauro- and glyco-chenodeoxycholic acids (suggesting increased deconjugation by luminal bacteria). **Conclusions:** iPLA2beta deficiency in aged mice caused biliary ductular expansion, and an accumulation of bile acids within enterohepatic circulation. This accumulation was a result of sup-

pressed hepatic and intestinal bile acid export and increased bile acid syntheses, and the latter may lead to a decreased level of the bile acid precursor cholesterol. Thus, iPLA2beta plays a homeostatic role by regulating hepatic bile acid metabolism affecting systemic cholesterol levels and intestinal bile acid contents.

3.11 Delayed intrahepatic cholestasis induced by anabolic steroids in a patient with haploinsufficiency of the pregnane X receptor (PXR/NR1I2)

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Case description: We describe a 32 year old male patient who developed cholestatic liver injury two weeks after a 2-month course of anabolic steroids. **Methods and results:** Next generation sequencing of a panel of 24 cholestasis-related genes revealed a heterozygous 2 basepair deletion in exon 1 of the pregnane X receptor (PCR/NR1I2) gene. Analysis of serum bile salts revealed marked imbalances, strongly resembling the changes observed in patients with biliary obstruction (BO). PXR haploinsufficiency coupled to AAS severely reduced the secretion of bile, causing massive accumulation of taurine- and glycine-conjugated primary bile acids. Absence of secondary BAs such as DCA and LCA in his plasma is consistent with an impaired biliary secretion. LCA was absent from the PXR sample, its 3-glucuronide derivative presented an impressive 112-fold accumulation; while the same accumulation was limited to 7.7-fold in BO samples. **Conclusions:** PXR haploinsufficiency reveals a network of transcriptional regulatory functions activated in the liver under xenobiotic stress, which appears to require two functional copies of the gene. Deranged bile salt levels following anabolic steroid consumption outline the central role of PXR in bile acid synthesis, modification and export. Comparison of BA levels found in the serum of the PXR/NR1I2 haploinsufficient individual with patients suffering from biliary obstruction of other sources indicate subtle but significant differences in glucuronidation. These findings suggest a more central and crucial role of PXR/NR1I2 in the hepatic handling and clearance of bile acids than has been previously suggested.

3.12 The power of NGS: Results from a dedicated sequencing panel of 24 genes in 40 patients with cholestatic liver disease

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Background: Cholestasis indicates bile secretory failure, which might be caused by environmental factors and gene variants, or combination of both. Although there is variability in underlying causes of hepatocellular cholestasis, many patients develop cholestasis of unknown etiology and gene mutations of critical hepatobiliary transporters or their regulators may be suspected. Our aim now was to employ next generation sequencing (NGS) to dissect genetic risk factors in adult patients with cholestatic liver injury of unknown etiology. **Methods:** The full length coding sequences of 24 genes known to be involved in regulation and transport of drugs and metabolites into bile (Table 1) were determined by NGS sequencing in selected patients with unexplained cholestatic liver injury, as defined by an at least two-fold increase of serum alkaline phosphatase (AP) activities and ALT/AP ratio ≤2. Population frequency and biochemical impact of all detected variants were analysed; rare, high-impact variants were also considered.

Tab. 1: Cholestasis sequencing panel:

ABCB4	ABCB11	ABCC2	ABCG5	ABCG8
ATP8B1	CIRH1A	CLDN1	JAG1	NOTCH2
NR1H4	NR1I2	UGT1A1 – 10	VIPAR	VPS33B

Results: Sequence analysis of 24 genes in 40 patients with cholestatic liver disease revealed heterozygosity for known disease-associated variants in 12 patients, and new high-impact potential candidate variants (splice site -1 or -2, frameshift deletions, rare non-conservative variants) in altogether 22 patients. Only 8/40 (20%) patients showed no credible variants. **Conclusions:** A NGS panel of 24 cholestasis-related genes resulted in detection of creedal candidate gene variants in approximately three quarter of all index cases analysed. Only 4 of the 24 genes analysed have revealed no candidate variant to date. We are in the process of designing the next panel iteration by adding new candidate genes associated with cholestatic liver disease (CFTR, TJP2, GBPAR and others) and removing genes that have failed to turn up any candidate variants to date. Thus we are hoping to identify the underlying genetic cause of cholestasis in at least 90% of all cases analysed.

3.13 Differential modulation of vesicular and non-vesicular associated microRNAs isolated from sera of partially hepatectomized rats

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Cell-free circulating microRNAs are protected from degradation by their association with either component of the RNAi machinery or vesicles. Although increasing evidences shows that different types of vesicles are capable of transporting microRNAs, current research mainly focuses on the characterization of exosomal microRNAs. Reason for this is that exosomal-microRNAs are thought to directly participate in intercellular communication. However, is yet unclear whether exosomal-microRNAs are also the most reliable source for discovering disease-associated biomarker. In this study, the distribution of circulating microRNAs associated to either the vesicular or non-vesicular fractions of sera isolated from partially hepatectomized rats was measured. Here we show that independently from their origin, levels of cell-free miR-122, miR-192, miR-194 and Let-7a are upregulated two days after partial hepatectomy. The inflammation-associated miR-150 and miR-155 are upregulated in the vesicular-fraction only, while the regeneration-associated miR-21 and miR-33 are upregulated in the vesicular- and downregulated in the non-vesicular fraction. Our study shows for the first time the modulation of microRNAs contained in the non-vesicular fraction. Overall, these findings suggest that, in the search for novel disease-associated biomarkers, the investigation of either vesicular or non-vesicular microRNAs may be more informative compared to the analysis of microRNAs isolated from unfractionated serum.

3.14 Effect of paper industry leachate on various serological indices and serum proteins

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Background & Aims: Pulp and paper industry consumes more resources as compared to any other industry around the globe. The toxicity of waste water (leachate) resulting from paper processing has become a serious issue now a days. So, current research was aimed to assess various toxic aspects of pulp and paper industry waste water. **Methods/ Study design:** Study design involved the division of Wistar rats (n = 5) of about 245 ± 5 g in three groups viz, Control group (4 ml/kg normal saline), Group 1 (4 ml/kg leachate) and Group 2 (4 ml/kg 1:10 diluted leachate). All the animals received the treatment through intraperitoneal injection. After 24 h of the injection, all the animals were sacrificed and blood was collected, sera were separated and processed for further analysis. **Results:** Serological analysis revealed that leachate induction leads to significant positive variations in level of serum aspartate aminotransferases (AST) ($P < 0.0001$), cholesterol ($P < 0.0001$) and High density lipoproteins (HDL) ($P < 0.0001$) while a significant negative change in triglycerides ($P = 0.0002$) and creatinine ($P = 0.0370$) level in both experimental groups. Alanine aminotransferases (ALT) level showed a significant increment in Group 1 and a decrement in Group 2 compared to control group ($P < 0.0001$). SDS page analysis revealed an overall decreased expression of various proteins in both experimental groups. The protein fractions of 317, 212, 178, 134, 89, 74 and 68 KDa were found in Group 1 compared to control (331, 215, 180, 139, 112, 91, 75 and 69 KDa). However, protein fraction of 202, 171, 136 and 91 KDa were present in

Group 2 compared to control. **Conclusion:** These findings confirmed that paper industry leachate is extremely noxious and induce alterations in various serological parameters and also interfere with the expression of proteins.

3.15 Hepatic gene expression profile characterizes high levels of liver regeneration related genes in nonalcoholic fatty liver disease

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Nonalcoholic fatty liver disease (NAFLD) is a chronic hepatic disease caused by abnormal or excessive accumulation of fat in hepatocytes. NAFLD covers a spectrum of liver disorders ranging from simple steatosis (S) to nonalcoholic steatohepatitis (NASH) and cirrhosis, and is associated with changes in gene expression profiles. Tissue gene expression profiling has been precious for developing diagnostic and predictive biomarkers as well as for improving therapeutical strategies in many diseases. Therefore, the aim of this study was to use microarray analysis to characterize a liver gene expression profile that differentiates individuals diagnosed with steatosis or steatohepatitis. The discovery cohort (n = 22) including well-characterized human liver samples, categorized as normal (n = 7), steatosis (n = 7), and nonalcoholic steatohepatitis (n = 8), was analyzed by Affymetrix GeneChip and qRT-PCR. A gene set enrichment analysis was performed to identify processes that distinguish between steatosis/NASH and normal groups and hence, might be novel biomarkers or treatment targets. Among 1624 genes, Gene Ontology (GO) analysis identified six genes, involved in tissue repair/regeneration, which were differentially expressed and significantly upregulated in patients with NASH. This subset of the top-regulated liver regeneration-related genes includes amphiregulin (AREG), plasminogen activator receptor urokinase-type (PLAUR), thrombospondin 1 (THBS 1), chemokine CC ligand 20 (CCL20), aldo-keto reductase family 1 member B10 (AKR1B10) and interleukin 32 (IL-32). These findings were validated in a second, independent and large cohort (n = 112) and confirmed by quantitative real-time PCR (qRT-PCR). Patients with NASH (n = 43) had significantly higher levels of AREG, PLAUR, THBS 1, CCL20, AKR1B10 and IL-32 expression compared to steatotic (n = 37) and/or normal (n = 32) liver tissue. Subsequently, in this study we aim to correlate the mRNA expression of these genes with steatosis grade, NASH activity score, fibrosis, adipositas, BMI, Diabetes Mellitus and hypercholesterolemia. In conclusion, hepatic gene expression profiling identified a set of liver regeneration-related genes as novel targets that can be exploited to improve diagnosis and treatment of patients with NAFLD.

3.16 Hsp72 overexpression protects from drug-induced- and lipotoxic liver injury

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Introduction: Heat shock protein (Hsp) 72 is a molecular chaperone that is upregulated in a response to a variety of stress situations and possesses broad cytoprotective functions. The hepatic function of Hsp72 remains largely unknown. **Aims & methodology:** To study the importance of Hsp72 in the liver, we generated transgenic mice overexpressing Hsp72 under the control of a tissue-specific tetracycline-inducible system and crossed them 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). Long-term feeding (8 weeks) with methionine choline-deficient diet (MCD) was used to induce lipotoxic liver damage. **Results:** Hsp72-LAP mice displayed doxycycline-regulated, robust Hsp72 overexpression in hepatocytes, but not in the other tissues or cell types. Eighteen hours after acetaminophen injection, a significantly lower liver injury was noted in Hsp72-LAP mice in comparison to single transgenes (ALT: 933 vs. 1977, $p < 0.05$). A trend towards a faster clearance of APAP from the serum was seen in Hsp72-LAP mice 4 h after the injection (180 vs. 243, $p = 0.05$), Overexpression of Hsp72 protected also protected from formation of APAP protein adducts ($p = 0.03$) and JNK hyperphosphorylation. After MCD-feeding, Hsp72-LAP mice displayed lower ALT levels (105 vs. 225, $p = 0.02$) as well as decreased JNK phosphorylation and RIP-3 activation. While overexpression of Hsp72 did not affect the extent of MCD-diet induced steatosis, it resulted in higher phosphatidylcholine levels ($p = 0.04$) and a trend towards a lower C 18 / C 16 ratio ($p = 0.08$). **Conclusions:** Our results suggest that Hsp72 overexpression protects against specific types of liver injury.

3.17 Identification of cytochrome CYP2E1 as critical mediator of synergistic effects of alcohol and cellular lipid accumulation in hepatocytes in vitro

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Clinical studies propose a causative link between the consumption of alcohol and the development and progression of liver disease in obese individuals. However, it is incompletely understood how alcohol and obesity interact and whether the combined effects are additive or synergistic. The aim of this study was to establish an in vitro model for joint effects of alcohol and steatosis in hepatocytes in vitro. **Methods and Results:** Lipid accumulation in primary human hepatocytes (PHH) was induced by incubation with fatty acids. Subsequently, steatotic and control hepatocytes were incubated with up to 50 mM alcohol. This alcohol concentration on its own revealed only minimal effects but significantly enhanced fatty acid induced lipogenesis and cellular triglyceride content compared to control cells. Similarly, lipid peroxidation, oxidative stress and pro-inflammatory gene expression, as well as CYP2E1 levels and activity were synergistically induced by alcohol and steatosis. CYP2E1 inhibition blunted these synergistic pathological effects. Notably, alcohol and cellular steatosis also induced autophagy in a synergistic manner, and also this was mediated via CYP2E1. Further induction of autophagy ameliorated the joint effects of alcohol and free fatty acids on hepatocellular lipid accumulation and inflammatory gene expression while inhibition of autophagy further enhanced the dual pathological effects. Further analysis revealed that combined stimulation with alcohol and free fatty acids induced JNK-activation and JUN-phosphorylation significantly more than either of the two stimuli alone. Conversely, JNK inhibition abrogated the induction of autophagy in steatotic hepatocytes by alcohol. **Conclusion:** In summary, our data indicate that alcohol induces not only pathological but also protective mechanisms in steatotic hepatocytes via CYP2E1 mediated JNK induction. These findings may have important implications on the prognosis and treatment of alcoholic liver disease particularly in obese individuals.

3.18 Innate Immune Cell Activation in a Human in-vitro Model of Nonalcoholic Steatohepatitis (NASH)

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Background: The immune system likely plays a significant role in the pathogenesis of fatty liver disease. The metabolic changes induce immunological responses resulting in nonalcoholic steatohepatitis (NASH) and further aggravation of the metabolic derangement in a feed-forward loop. However, most of the evidence regarding the immune system role in the context of NASH is based on the use of mouse models, which makes it difficult to rely on these conclusions for humans. Therefore, we aimed in this study to analyze the activation and function of several liver resident innate cell populations in a human in-vitro cell culture model of steatosis. **Methods:** Intrahepatic lymphocytes (IHLs) and peripheral blood mononuclear cells (PBMCs) were isolated from patients

undergoing liver resections. As a human in vitro model for steatosis, fully differentiated HepaRG cells were treated with 0.5 mM free fatty acids (FFAs) at a 2:1 ratio of oleate (OA) and palmeate (PA) for 24 h. Induction of steatosis was confirmed by oil red O staining. FFA-treated HepaRG cells were co-cultured with or without IHLs and PBMCs. Expression levels of activation markers such as CD69 and HLA-DR on natural killer (NK), mucosal-associated invariant T (MAIT) and invariant natural killer T (iNKT) cells were assessed by multicolor flow cytometry. **Results:** While intrahepatic NK cells were not activated in response to co-culture with FFA-treated HepaRG cells, MAIT cells showed an increased frequency and expression level of CD69 (75 vs. 79%; P = 0.03 and MFI 1974 vs. 2207; P = 0.03) as well as HLA-DR (22 vs. 30%; P = 0.04 and MFI 733 vs. 1038; P = 0.03). Remarkably, intrahepatic invariant NKT cells were activated after co-culture with FFA-treated HepaRG cells with an increased frequency and expression level of CD69 (68 vs. 86%; P = 0.03 and MFI 1345 vs. 1931; P = 0.03) as well as HLA-DR (31 vs. 51%; P = 0.04 and MFI 603 vs. 1410; P = 0.03). Peripheral blood iNKT cells were activated to a lesser extent with an increased frequency and expression level of HLA-DR (9 vs. 15%; P = 0.03 and MFI 265 vs. 827; P = 0.03). Activation of iNKT cells was diminished when lymphocytes were separated from FFA-treated HepaRG cells by a transwell system, indicating that cell-to-cell contact was necessary for activation. Next, production of cytokines was assessed by intracellular staining and flow cytometry after an additional 2 h of stimulation with αGalCer in the presence of brefeldin A. After co-culture with FFA-treated HepaRG cells, iNKT cells were more frequently positive for the pro-inflammatory Th1 cytokine IFNγ. **Conclusion:** Collectively, these data show that invariant NKT cells are activated in response to FFA-treated HepaRG cells by direct cell-to-cell contact, leading to increased secretion of the Th1 cytokine IFNγ, which could contribute to the pro-inflammatory environment within a NASH liver.

3.19 Insulin-induced cytokine production as potential contributor to hepatic insulin resistance

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Overweight or obese patients try to compensate their insulin resistance by an enhanced production of insulin in pancreatic beta-cell. Since pancreatic hormones are released into the portal circulation, liver is exposed to very high concentrations of insulin over prolonged periods in these patients. There is evidence that insulin might enhance the inflammatory response to e.g. endotoxin in immune cells. Therefore, the hypothesis was tested that insulin per se might trigger the synthesis of pro-inflammatory cytokines in macrophages. These cytokines in turn might render hepatocytes insulin-resistant. Human U937 cells were differentiated into macrophages and stimulated with 100 nM insulin for 24 h. The induction of cytokine expression was determined by qPCR and Western blot. Primary cultures of rat hepatocytes were incubated with supernatants of insulin-treated U937 macrophages and the activation of the insulin receptor signal chain in these hepatocytes was recorded by qPCR of insulin-induced genes and Western blot of signal chain proteins. Insulin induced the expression of IL-1β in U937 macrophages about 2.5-fold both on the mRNA and protein level. In contrast to the LPS-dependent IL-1β induction, the insulin-dependent IL-1β induction was not affected by polymyxin B. In U937 macrophages insulin induced the phosphorylation of IKKβ and IκB. The insulin-dependent IL-1β induction was abolished by the IKKβ inhibitor TPCA-1, indicating that insulin triggered the IL-1β formation by an activation of NFκB. Apart from IL-1β insulin induced the expression of OSM and IL-8. In hepatocytes that were incubated with supernatants of insulin-treated U937 macrophages the insulin-dependent glucokinase induction was reduced by 50%. This could be attributed to an inhibitory serine phosphorylation of the insulin receptor substrate either by an IL-1β-dependent activation of IKKβ or by an OSM-dependent activation of ERK1/2. In addition, an OSM-dependent STAT3 activation and SOCS3 induction was observed that might have contributed to the interruption of the insulin receptor signaling chain in hepatocytes. An insulin-dependent activation of macrophages might thus contribute to the development of insulin resistance in the liver.

3.20 Kombinierte Aktivitäten von JNK1 und JNK2 in Hepatozyten schützen vor Toxin-induzierten Leberschädigung

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Hintergrund & Ziele: Hepatozytisch c-Jun N-terminale Kinases (JNK) 1 und JNK2 haben überlappende und verschiedene Funktionen. Nach acetaminophen- oder carbon tetrachloride (CCl4)-induzierten Leberschädigung sind JNK-Proteine durch Phosphorylierungen aktiviert und die Höhe der Aktivierung korreliert mit dem Grad der Schädigung. Es ist schon bekannt, dass der JNK Inhibitor SP600125 acetaminophen-induzierten Leberschädigung blockieren könnte. Hier, wir untersuchten die Rolle von JNK in medikamenten-induzierten Leberschädigung (DILI) in Lebergewebe von Patienten und bei Mäusen mit genetischer Hemmung von JNK in Hepatozyten. **Methoden:** Wir untersuchten Leberschnitte von Patienten mit DILI (aufgrund Phenprocoumon, nicht-steroidale entzündungshemmende Medikamente, Paracetamol, Autoimmunhepatitis oder Patienten ohne akutes Leberversagen (Kontrollen)). Gesamt und phosphoryliertes JNK wurden immunhistochemisch untesucht und Immunoblot-Assays durchgeführt. Mäuse mit Hepatozyten-spezifische Deletion von Jnk1 (Jnk1Δhepa) oder eine Kombination von Jnk1 und Jnk2 (JnkΔhepa) sowie Jnk1-floxed C57BL/6 (Kontrolle) Mäuse wurden wiederholt mit injiziert, um Fibrose oder mit Acetaminophen eine toxischen Leberschädigung auszulösen. Wir führten Genexpression Microarray und Phosphoproteomic Analyse die Mechanismen der JNK-Aktivität in Leberzellen zu bestimmen. **Ergebnisse:** Leberproben von Patienten mit DILI enthalten mehrere aktivierten JNKs vorwiegend in Hepatozyten im Vergleich zu gesundes Gewebe. Die Applikation von Acetaminophen in JnkΔhepa Mäusen führte zu einer erhöhten Schädigung der Leber im Vergleich zu Jnk1Δhepa oder Kontrollmäusen, bezogen auf Serumwerte und der Analyse von mikroskopischen und histologischen Lebergeweben. Die Injektion von CCl4 induzierte deutlich stärker Leberschädigung in JnkΔhep Mäusen, basierend auf erhöhte Entzündung, Zellproliferation, und Fibrose Progression im Vergleich zu Jnk1Δhepa oder Kontrollmäusen. Hepatozyten aus Paracetamol-behandelten JnkΔhepa Mäusen zeigten eine erhöhte oxidative Stress-Reaktion, sowie eine verminderte Aktivierung von AMPK und JunD und Nekrose. Die Nutzung von SP600125 vor oder mit Paracetamol schützt JnkΔhepa und Kontrollmäusen vor einer Leberschädigung. **Schlussfolgerungen:** Die gemeinsamen Funktionen der JNK1 und JNK2 in Hepatozyten schützen vor CCl4- und Paracetamol-induzierten Leberschädigung. JNK-Hemmung mit SP600125 hat Nebeneffekte.

3.21 Verlust von Caspase 8 in Leberparenchymzellen schützt vor Obstruktiver Cholestase

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Hintergrund & Ziele: Gallengangsobstruktion und Akkumulation toxischer Gallensäuren sind wichtige Kennzeichen der cholestatische Lebererkrankung (z.B.: PBC, PSC), die massiven Hepatozyten Zelltod verursacht. Caspase 8 (CASP8) ist ein essentieller Todesrezeptor für Apoptose-Aktivität, und daher könnte seine Modulation wichtig für die Pathogenese von obstruktiven cholestatischen Leberschädigung sein. Unsere Hypothese ist, dass CASP8 in Leberparenchymzellen eine wichtige Rolle in einem Mäusemodell der experimentellen Cholestase spielt. **Methoden:** Leberschädigung wurde in einer Kohorte von menschlichen Seren und Biopsien von PBC-Patienten (n = 28) untersucht. Parallel dazu wurden Hepatozyten-spezifischen CASP8 Knockout-Mäusen (Casp8Δhepa) durch Kreuzung von Mäusen, die loxP-Ort-flankierten (Casp8f/f) Allele

des Gens mit ALFP-cre transgene Mäusen, generiert. Kontrolle Casp8f/f und Casp8Δhepa Mäuse wurden chirurgische Ligation des Choledochum (BDL) für 28 Tage ausgesetzt. **Ergebnisse:** Patienten mit PBC zeigten typischen Symptome (Müdigkeit und Juckreiz) und cholestatischen Muster in Leberfunktionstests (LFT), mit einem hohen Grad der alkalischen Phosphatase, Transaminasen, Cholesterin sowie erhöhte Titer von antimitochondrialen Antikörpern. Verschärft gespalten Caspase-3-Enzymaktivität und die Überexpression von RIP3 war charakteristisch für Leber Explantate von PBC-Patienten im Vergleich zu gesunden Kontrollpersonen, begleitet von erhöhten Proteinspiegel von TNF und IL-6. Casp8Δhepa nach BDL zeigten eien verringerte Anzahl und Größe der Nekroseherde im Vergleich zu Casp8f/f Mäusen. Signifikant verringert Serum-Alanin (ALT) und Aspartat (AST) Transaminasen, TUNEL-Färbung und cleaved Caspase-3 und Cytochrom C-Proteinexpression wurden in Casp8Δhepa Mäusen 28 Tage nach BDL gefunden, sowie eine verringerten Ausgleichsproliferation (Ki-67, PCNA) und duktularen Reaktion (CK-19). Diese Ergebnisse wurden in einem verminderten Entzündungsprofil durch geringere IL-6, TNF-α-Proteinspiegel ausgelöst und durch das Eindringen von F4/80+, CD11b+ und CD45+ Populationen verringert. Insgesamt wurde die Leberfibrose (Sirius red, Collagen IA1, ASMA) deutlich in Casp8Δhepa verbessert. Mechanistisch verringerte Aktivierung von JNK, RIP1 und RIP3 korreliert mit dem gezeigten Schutz nach chronischen BDL. **Schlussfolgerung:** CASP8 in Hepatozyten ist ein wesentlicher Orchestrator cholestatischer Leberschädigug und deren spezifische Modulation könnte ein interessantes pharmakologisches Ziel sein, das in der Klinik zur Behandlung von obstruktiven Lebererkrankung verwendet werden kann.

3.22 Lysophosphatidylcholine (LPC) as central player for control of hepatocellular fatty acid influx

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Background: In NASH the intracellular ratio of phosphatidylcholine (PC): lysophosphatidylcholine (LPC) is decreased due to activation of phospholipase A2. Responsible is the calcium independent membrane phospholipase A2 (iPLA2β) which is part of the heterotetrameric fatty acid uptake complex consisting also of FABPPM, CD36 and caveolin1. The bile acid-phospholipid conjugate UDCA-LPE inhibits fatty acid influx by interference with iPLA2β. **Aim:** Evaluation of the metabolic consequences of iPLA2β inhibition by UDCA-LPE in HepG2 cells. **Methods:** In HepG2 cells the bile acid-phospholipid conjugate ursodeoxycholate-lysophosphatidylethanolamide (UDCA-LPE) as iPLA2β inhibitor was used to modify intracellular LPC levels. We examined the impact of LPC on p-JNK1 and transcription of the heterotetrameric fatty acid transport complex constituted of CD36, FABPPM, caveolin1 and iPLA2β. **Results:** Inhibition of iPLA2β by UDCA-LPE resulted in suppression of cytosolic lysophosphatidylcholine (LPC) which was accompanied by a corresponding decrease of phosphorylated JNK1. Low pJNK1 suppressed the synthesis of all four members of the fatty acid uptake complex. Thus, the complex faded from the detergent resistant plasma membrane fraction explaining the inhibition of fatty acid influx in hepatocytes. The role of LPC as inducer of pJNK1 was supported by in vitro addition of 1 – 10 µM LPC to delipidated cytosolic extracts. It resulted in pJNK1 dependent synthesis stimulation of the fatty acid uptake complex. **Conclusion:** The generation of LPC controls via JNK1 the constitution of the membrane fatty acid uptake complex.

3.23 Modelling of human nonalcoholic fatty liver disease with hepatocyte like cells derived from pluripotent stem cells

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Nonalcoholic fatty liver disease (NAFLD) is an increasingly common diagnosis in the Western Hemisphere. It is defined by an accumulation of lipid droplets in more than 5% of hepatocytes. In the beginning the disease is rather benign, but later on patients develop steatohepatitis, cirrhosis and up to 27% of these patients end up with hepatocellular carcinoma. The molecular reasons of this disease are still questioned, but it is well-recognised that NAFLD is strongly associated with obesity and insulin resistance. In this metabolism-based field of research, results obtained from rodent model systems cannot be easily extended to humans as both organisms differ in their metabolisms. Unfortunately, liver cells from steatosis patients are very rarely available and not suitable for longer experiments as hepatocytes rapidly dedifferentiate in culture. Therefore, we have established a human model system for NAFLD based on hepatocyte like cells (HLCs) generated from pluripotent stem cells. We are able to induce the accumulation of lipid droplets (LDs) in these cells by adding oleic acid (OA) into the medium. LD formation has been documented by staining with Oil Red O or BODIPY. After fat induction with OA the expression of PLIN2, a protein covering LDs, was consistently upregulated. As PLIN2 knockout mice are protected against the development of steatosis, we selected PLIN2 expression as a molecular marker for the successful induction of steatosis. We thoroughly investigated the consequences of LD accumulation on the level of gene expression. We found that many GO categories related to lipid, glucose and sterol metabolism were up-regulated in HLCs after OA induction. Interestingly, many members of the Peroxisome proliferator-activated receptor (PPAR) pathway, which is important for the regulation of lipid metabolism, were upregulated after fat induction. Modelling PPARα in HLCs with small molecules resulted in profound gene expression changes. Inhibition of PPARα with GW6471 resulted in down-regulation of genes involved in lipid catabolism, while activation via Fenofibrate reduced expression of AGPAT2 and HMGCR, which are involved in biosynthesis of phospholipids and cholesterol, respectively. Also insulin signalling was affected by PPARα modulation. Although obesity and NAFLD are increasing health problems worldwide, there is no specific treatment for NAFLD at the moment. Our HLC-based NAFLD model can be used in the future to screen for drugs that might interfere with LD accumulation.

3.24 Nuclear ErbB2 Expression of Hepatocytes in Alcoholic Steatohepatitis as Response to Cellular Stress Events

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Background: ErbB2 is a prominent member of the epidermal growth factor receptor superfamily, a group of transmembrane receptors that mainly attract attention 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. **Methods:** The immunohistochemical pattern of ErbB2 staining was analyzed in 334 liver biopsy samples from patients with hepatic dysfunction. **Results:** We found a cytoplasmic and nuclear ErbB2 expression in hepatocytes from different disease conditions, with the strongest expression being detected in alcoholic steatohepatitis. These ErbB2 overexpressing hepatocytes revealed peculiar metabolic and hormonal changes: enhanced lipid turnover (increase of fatty acid synthase and acyl-CoA-dehydrogenase), downregulation of estrogen receptor, cell cycle alterations (increase of p21 and heat shock protein 27), and an increase of phospho-Stat3, a downstream effector of nuclear ErbB2 signaling. Notably, ballooned hepatocytes, often interpreted as degenerative cells, exhibited a particularly strong ErbB2 expression and appeared to be highly metabolically active as described above. Of note, an increased ErbB2 expression as well as metabolic alterations and signs of cell cycle deregulation similar to that described in human steatohepatitis specimens were detected when HepG2 cells were subjected to 48-hour ethanol treatment. **Discussion:** These novel observations on hepatocellular ErbB2 expression with evidence of nuclear receptor signaling and metabolic alterations imply a so far unknown mechanism in hepatocytes upon cellular stress events, particularly ethanol exposure. This molecular reprogramming might improve cellular survival, have a significant effect on disease course with therapeutic implications and play a role in progression to hepatocellular carcinoma and thus opens a wide field of future research on the role of hepatocellular ErbB2 expression.

3.25 Regulation des Energiestoffwechsels in Hepatozyten durch Morphogene

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Mitochondrien sind komplexe Organelle, die an unterschiedlichen zellulären Signalwegen und Stoffwechselaktivitäten beteiligt sind. Die wichtigsten Stoffwechselfunktionen der Mitochondrien sind dabei zum einen,

die zentrale Steuerung des metabolischen Stoffflusses, wie der Citratzyklus und die β-Oxidation der Fettsäuren und zum anderen die Generierung von Energie in Form von ADP mittels oxidativer Phosphorylierung. Für die Regulation des Energiestoffwechsels in Hepatozyten durch Morphogene untersuchten wir eine Mausmodell, in dem ein hepatozytenspezifischer Knock-out (Ko) für Smoothened (Smo), ein wichtiges Transduktorprotein des Hedgehog (Hh) Signalweges, generiert wurde. Der Hh-Signalweg zählt zu den Morphogenen und spielt eine wichtige Rolle in der Organogenese und Tumorgenese. Unsere neuesten Erkenntnisse zeigten, dass der Hh-Signalweg ein aktiver Signalweg im adulten Gewebe ist und eine entsprechende Auswirkung auf die endokrine Funktion der Leber besitzt sowie im Fettstoffwechsel beteiligt ist [1]. Für einen Einblick in den Energiestoffwechsel lagen unsere Schwerpunkte in der Untersuchung des Sauerstoffverbrauches der Hepatozyten, den ATP-Gehalt in der Zelle sowie einzelner Gene, welche mitochondriale Proteine kodieren wie Untereinheiten der Atmungskettenkomplexe, der β-Oxidation und Entkoppelungsproteine. Für die Untersuchungen der mitochondrialen Atmung wurde der Sauerstoffverbrauch der hepatischen Mitochondrien im gekoppelten und entkoppelten Zustand am Oxygraphen-2k Oroboros vorgenommen. Der ATP-Gehalt wurde mit dem CellTiter-Glo® Luminescent Cell Viability Assay gemessen und die Untersuchung des mRNA-Expressionslevel erfolgte über eine quantitative real-time PCR. Die ersten Messungen der mitochondrialen Atmung zeigen, dass die Hepatozyten der Ko-Tiere eine geringere Sauerstoffkonzentration und somit einen langsameren Sauerstoffverbrauch gegenüber den WT-Tieren besitzen. Außerdem zeigte sich in Hepatozyten der Ko-Tiere ein drastischer Abfall der ATP-Konzentration. Dahingegen konnte eine signifikante Steigerung der Expressionslevel der F1-Einheit der ATP-Synthase, der Schrittmacherenzyme der β-Oxidation und im Entkopplungsprotein 2 beobachtet werden. Diese Ergebnisse lassen darauf schließen, dass der Hh-Signalweg einen Einfluss auf den Energiestoffwechsel hat und somit auf die mitochondrialen Funktion. Um einen tieferen Einblick in die vorliegenden Mechanismen zu erhalten, sind zukünftig Messungen des mitochondrialen Membranpotentials und mitochondrialer Masse mittels Facs geplant sowie weiterführende Analysen hinsichtlich der Rolle der Entkopplungsproteine 1 und 3. [1] Matz-Soja et al., Hepatic Hedgehog signaling contributes to the regulation of IGF1 and IGFBP1 serum levels. Cell Commun Signal. 2014, 18;12:11.

3.26 Regulation of Plasma Membrane Localization of the Na+-taurocholate cotransporting polypeptide (Ntcp) by Hyperosmolarity and Tauroursodeoxycholate

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Introduction: In perfused rat liver, hepatocyte shrinkage induces a Fyn-dependent retrieval of the bile salt export pump (Bsep) and multidrug resistance-associated protein 2 (Mrp2) from the canalicular membrane leading to cholestasis. However little is known about the effects of hyperosmolarity on short-term regulation of the Na+-taurocholate cotransporting polypeptide (Ntcp), the major bile salt uptake system at the sinusoidal membrane of hepatocytes. The aim of this study was to analyze hyperosmotic Ntcp regulation and the underlying signaling events. **Methods:** Rat livers were perfused with either normo- or hyperosmotic Krebs-Henseleit buffer. Addition of inhibitors, Db-cAMP and tauroursodeoxycholate (TUDC) to the influent perfusate was made by dissolution into the buffer. Liver tissue was obtained for immunofluorescence Ntcp- and Bsep-staining following analyzation of transporter localization. Cell culture experiments were performed in primary rat hepatocytes and HepG2 cells. Liver tissue was also processed for immunoprecipitation and Western blot analysis of Src kinases and Ntcp. Generation of ROS was measured using H2DCFDA. **Results:** Hyperosmolarity induced a significant retrieval of Ntcp from the basolateral membrane, which was accompanied by an activating phosphorylation of the Src-kinases Fyn and Yes, but not of c-Src. Hyperosmotic internalization of Ntcp was sensitive to SU6656 and PP-2, suggesting that Fyn mediates Ntcp-retrieval from the basolateral membrane. Hyperosmotic internalization of Ntcp was also found in livers from wildtype mice, but not in p47phox knockout mice. Paralleling the inhibition of ROS formation, the hyperosmolarity-induced Fyn phosphorylation was sensitive to inhibition of PKCζ by the specific PKCζ pseudosubstrate, and by the broad spectrum PKC inhibitors chelerythrine and Gö 6850. TUDC and cAMP reversed hyperosmolarity-induced Fyn activation and triggered re-insertion of the hyperosmotically retrieved Ntcp into the membrane. This was associated with dephosphorylation of the Ntcp on serine residues. Insertion of Ntcp by TUDC was sensitive to the integrin inhibitory hexapeptide GRGDSP and inhibition of protein kinase A. In line with a TUDC-induced reversal of hyperosmotic Fyn activation, TUDC not only prevented the hyperosmolarity-induced Ntcp retrieval from the plasma membrane, but also the hyperosmolarity-induced Bsep retrieval from the canalicular membrane. **Discussion/Conclusion:** These findings suggest a coordinated, oxidative stress- and Fyn-dependent retrieval of sinusoidal and canalicular bile salt transport systems from the corresponding membranes. Ntcp insertion was also identified as a novel target of β1-integrin-dependent TUDC action, which is frequently used in the treatment of cholestatic liver disease. The study provides new insights into the regulation of bile salt transport.

3.27 Sequencing of ATP8B1, ABCB11 and ABCB4 revealed 135 genetic variants in 374 unrelated patients with suspected intrahepatic cholestasis

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ATP8B1, ABCB11, and ABCB4 encode the aminophospholipidflippase familial intrahepatic cholestasis 1 (FIC1), the bile salt export pump (BSEP), and the phospholipidfloppase multidrug resistance protein 3 (MDR3), respectively, that play central roles in bile formation. Mutations in these transporters are associated with cholestatic liver diseases of varying severity ranging from milder forms like intrahepatic cholestasis of pregnancy (ICP), benign recurrent intrahepatic cholestasis (BRIC) or low phospholipid-associated cholelithiasis (LPAC) to progressive familial intrahepatic cholestasis (PFIC). At present, gene sequencing is the main method to investigate the genetic background of these different types of intrahepatic cholestasis. To confirm diagnosis based on the genetic background, 374 blood samples of unrelated patients presenting with a cholestatic phenotype of diverging manifestation were obtained from 14 different countries. Genomic DNA was used for sequencing analysis of all coding exons with surrounding intron regions of either ATP8B1, ABCB11 or ABCB4. For 81 patients, two or all three genes were sequenced. 27 variants including 6 new ones were detected in 119 samples of patients with assumed ATP8B1 (FIC1) deficiency. DNA sequencing from 187 patients with suspected ABCB11 (BSEP) mutations revealed 70 different variants, of which 36 represent novel variants. In 171 DNA samples, ABCB4 (MDR3) was analyzed which revealed 38 genetic variants comprising 18 novel ones. Nevertheless, in a variety of cases the genetic analysis uncovered only one heterozygous mutation for FIC1: 4/119 (3.4%), BSEP: 31/187 (16.6%), and MDR3: 27/171 (15.8%). Heterozygous mutations, common polymorphisms or synonymous variants probably not completely explain a severe cholestatic phenotype but should be considered as potential genetic factors for milder cholestatic diseases like BRIC, ICP or LPAC. In a number of patients without any grave mutation, common and/or synonymous variants were verifiable. The common FIC1 variants c.3531+8G>T and p.R952Q were detected in 39/105 (37.1%) samples. For BSEP, p.V444A combined with p.A1028A were demonstrated in 75/108 (69.4%) specimen. A combination of the three synonymous MDR3 variants p.L59L, p.N168N, and p.I237I was proven in 24/116 (20.7%) patients with no other mutation. In the described patient population, 135 genetic variants were detected in ATP8B1, ABCB11 or ABCB4 including numerous cases with only one heterozygous or even no single mutation. This demonstrates that other genes such as the TJP2 which was recently described by Sambrotta and colleagues in cases of low GGT cholestasis, as well as non genomic factors contribute to the phenotype of some of these patients. In our study, we focused on the common and synonymous genetic variants in ATP8B1, ABCB11 or ABCB4 which seem to have a significant effect on the development of a cholestatic phenotype.

3.28 Simvastatin senkt die hepatische Inflammation und Fibrose in Apolipoprotein E Knock-out Mäusen nach sieben Wochen Western Diät

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Einleitung: Nichtalkoholische Steatohepatitis (NASH) wird definiert als Auftreten von hepatischer Steatose und Inflammation, die mit zunehmender Fibrose zur Zirrhose fortschreitet. Kürzlich konnten wir beim murinen Apolipoprotein Knock-out (ApoE-/-) zeigen, dass die NASH wie beim Menschen mit dem metabolischen Syndrom einhergeht (Schierwagen, Maybüchen et al. 2015, Sci Rep). Beim Menschen werden Statine bereits breit im metabolischen Syndrom eingesetzt und deren pleiotrope Effekte werden zunehmend in der Leberfibrose untersucht. Diese Arbeit untersucht die molekularen Mechanismen und Effekte von Simvastatin (SMV) im murinen ApoE-/- NASH Modell. **Methoden:** ApoE-/- und Wild-Typ Mäusen wurde sieben Wochen Western Diät verabreicht. Aktiviertes SMV (RHOA/RAS/RAC1-Hemmer), NSC23766 (RAC1-Hemmer) oder Clostridium sordellii lethal toxin (RHOA-/RAC1-Hemmer) wurden über sechs Wochen kontinuierlich mittels osmotischer Mini-Pumpen subkutan injiziert. Die hepatische Steatose, Inflammation und Fibrose wurden mit RT-PCR, Western Blot, Histologie, sowie über die Messung von hepatischem Cholesterin und Hydroxyprolin bestimmt. **Ergebnisse:** Die hepatische Inflammation (F4/80 Färbung, pro-inflammatorische Marker auf RT-PCR Ebene) konnte nur nach SMV-Gabe signifikant gesenkt werden. Auch die hepatische Fibrose (Hydroxyprolin, Sirius Rot- und αSMA-Färbung, RT-PCR und Western Blot) wurde durch SMV-Gabe signifikant gemindert. Diese Effekte wurden sowohl durch Hemmung des RHOA-Signalwegs als auch durch Hemmung des RAS/RAF1/ERK-Signalwegs vermittelt. Simvastatin konnte nur milde Cholesterin-senkende Effekte im Serum von ApoE-/- Mäusen erreichen. Des Weiteren konnten in der Leber keine Verbesserungen der Steatose durch SMV-Gabe gezeigt werden. **Diskussion:** Simvastatin senkte die hepatische Inflammation und Fibrose, über die gleichzeitige Hemmung des RHOA/ROCK-Signalwegs und des RAS/ERK-Signalwegs. Der schwache Effekt auf die Steatose zeigt, dass dafür hauptsächlich die pleiotropen Effekte von Simvastatin verantwortlich sind. Patienten mit NASH könnten daher durch Gabe von SMV vor der Entwicklung einer schweren Fibrose geschützt sein.

3.29 TGR5 knockout mice are highly susceptible to LCA induced liver damage

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Introduction: TGR5 (Gpbar-1) is a G-Protein-coupled membrane-bound cell surface-receptor for bile acids associated not only with the regulation of bile acid homeostasis, but also with the suppression of macrophage activity (1,2). It is expressed in several different organs and cell types, e. g. nonparenchymal liver cells and monocytes/macrophages. Thereby, TGR5 can function as a mediator for bile acids in modulating different pathologies of the liver distinguished by inflammatory processes (1,2). Lithocholic acid (LCA) is a hydrophobic bile acid with toxic properties produced by intestinal bacteria. Feeding of a diet containing 1% LCA leads to intrahepatic cholestasis, inflammation and toxic liver damage in mice (3,4). The role of TGR5 during this process is yet to be determined. **Methods:** 8 – 12 week old mice with a TGR5 KO and WT genotype were sacrificed after feeding with a diet containing 1% LCA (lithocholic acid) for 4 days. Bile acid composition and concentration in serum and bile was determined. Serum analysis of liver enzymes and bilirubin was done using the Spotchem-biochemical analyzer. Depiction of the liver was performed utilising HE (hematoxylin-eosin) as well as immunohistochemical staining for proliferation and inflammatory markers. The mRNA levels of proinflammatory cytokines in the liver were determined by realtime-PCR. **Results:** As indicated by the sizes of necrotic areas in HE-staining and significantly higher elevated serum AST and bilirubin-levels, TGR5 KO mice were suffering from a more severe liver damage as compared to their wildtype littermates after being fed a diet containing 1% LCA for 4 days. The observed liver necrosis triggered by LCA was accompanied by an increase in mRNA of cytokines and chemokines. Furthermore, the TGR5 KO mice had a considerably enlarged bile acid pool with an altered serum bile acid composition. Proliferation of hepatocytes and cholangiocytes was significantly reduced in absence of TGR5 as determined by immunohistochemical staining for PCNA and Ki67. **Conclusion:** In conclusion TGR5 knockout mice suffer from more severe liver damage and also show reduced proliferative response after liver injury. This demonstrates a protective role of TGR5 in LCA-induced intrahepatic cholestasis. **References:** [1] Kawamata Y, Fujii R, Hosoya M, Harada M, Yoshida H, Miwa M, et al. A G protein-coupled receptor responsive to bile acids. J Biol Chem 2003;278:9435 – 9440. [2] Duboc H, Taché Y, Hofmann AF. The bile acid TGR5 membrane receptor: From basic research to clinical application. Digestive and Liver Disease 2014, Pages 302 – 312 [3] Woolbright BL, Li F, Xie Y, Farhood A, Fickert P, Trauner M, Jaeschke H. Lithocholic acid feeding results in direct hepato-toxicity independent of neutrophil function in mice. Toxicol Lett. 2014 Jul 3;228(1):56 – 66. [4] Fickert P, Fuchsbichler A, Marschall HU, Wagner M, Zollner G, Krause R, Zatloukal K, Jaeschke H, Denk H, Trauner M. Lithocholic acid feeding induces segmental bile duct obstruction and destructive cholangitis in mice. Am J Pathol. 2006 Feb;168(2):410 – 22

3.30 The impact of the interaction between Embryonic stem cell-expressed Ras and Arginase-1 in hepatic stellate cells

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Embryonic stem cell-expressed Ras (ERAS) represents a unusual member of the Ras family with remarkable characteristics in quiescent hepatic stellate cells (HSC).1 ERAS contains a unique N-terminal extension (38 amino acids) upstream of its guanosine triphosphate binding domain, which exhibits remarkable sequence deviations between human and rat species. However, the function of such an additional region with various motifs [1], which is not found in other classical Ras proteins (e. g., HRAS, KRAS and NRAS), remained unclear. Comparative proteome analysis revealed that ERAS is associated with 51 proteins (10 with the human ERAS, 3 with rat ERAS and 38 with both species), participating in various cellular processes, including cell cycle, transcription, immune response, signal transduction, cell adhesion, cytoskeletal dynamics and metabolism. The cytosolic Arginase-1 is one of these proteins, which is well-known to convert L-arginine to L-ornithine. Protein-protein interaction studies have shown that Arginase-1 physically binds to different ERAS variants, including isolated ERAS N-terminus, under cell-free condition using purified proteins. Preliminary results on deciphering new mechanisms controlling the ERAS signal transduction in quiescent HSC, including the interaction between ERAS and Arginase-1 will be discussed. [1] Nakhaei-Rad, S., Nakhaeizadeh, H., Kordes, C., Cirstea, I. C., Schmick, M., Dvorsky, R., Bastiaens, P. I., Haussinger, D. &Ahmadian, M. R. (2015). The function of embryonic stem cell-expressed Ras (E-Ras), a unique Ras family member, correlates with its additional motifs and its structural properties. J Biol Chem 290:15892 – 15903

3.31 The liver microcirculation might be important in promoting autoimmune hepatitis via maintaining an inflammatory cytokine milieu – A mathematical model study

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In autoimmune diseases, inflammatory cytokine concentrations are important for initiating and maintaining the status of autoimmunity. Autoimmune hepatitis (AIH) is an inflammatory liver disease characterized by a loss of immune tolerance against specific antigens located in hepato-

cytes. During the progression of the disease, antigen-presenting cells and different classes of T-helper cells secret specific cytokines important for maintaining the disease. As these cytokines are secreted into the local liver environment, the blood flow in liver sinusoids might influence the local cytokine concentration. Considering the liver tissue as a porous medium, based on Darcy's law, the microcirculation within a liver lobule was modelled. Using realistic physiological pressure differences and tissue permeabilities, the blood velocity inside the sinusoids could be calculated and validated with blood velocity data obtained via Orthogonal Polarization Spectral Imaging (OPSI). Furthermore, oxygen consumption is modelled to obtain Rappaport's acinus model. Finally, steady state spatial distributions of secreted cytokines within the liver lobule could be estimated for specified realistic production rates of T-helper cells. It could be demonstrated that the characteristics of the liver microcirculation might have an important impact on establishing inflammatory cytokine levels within the portal fields and the vascular septa promoting the occurrence of interface hepatitis.

3.32 The liver specific microRNA-122 modulates hepatic response to infection and inflammation by antagonizing YY1, FoxP3, Nfr1 and E2F4 molecular networks

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The liver specific microRNA miR-122 is essential for the maintenance of liver functionality. miR-122 deficient mice develop a pro-inflammatory phenotype in the liver, displaying an increased tendency in developing hepatocellular carcinoma (HCC). miR-122 transcription is regulated by Liver Enriched Transcription Factors (LETF). Transient inhibition of miR-122 activity results in the development of iron deficiency and decreases cholesterol biosynthesis. Expression of miR-122 is dysregulated in a number of human diseases including HCC, viral hepatitis, and NASH. Although a great number of miR-122 targets has been identified and validated, the global set of genes regulated by miR-122 and the actual function of this microRNA are still unknown. Consequently, how miR-122 modulates liver functionality in response to hepatic injury or during liver regeneration is not understood. Here we used polyribosomes fractionation on sucrose gradient in presence of cycloheximide to identify on genome wide level miR-122 target genes and identified that many of these genes are downstream to YY1, FoxP3, E2F4 and Nfr1 transcription factors. Remarkably, we also show that miR-122 transcription is regulated by the activity of the immunoresponsive cytokines TNFα, IL10, BMP6 and TGFβ. This indicates that miR-122 suppression of its targets is crucial in modulating the response of liver cells to infection and inflammation.

3.33 The platelet-derived chemokine CXCL4 exerts protective role in non-alcoholic steatohepatitis (NASH) in vivo

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Introduction: Non-alcoholic steatohepatitis (NASH) is the third most common reason for liver transplantations in developing countries and one of the fastest growing medical problems. Aim: It is known that platelets are involved in non-alcoholic fatty liver disease. The significance of CXCL4, one of the most abundant chemokines in platelets, in NASH development and progression remains unclear. We therefore investigated the role of CXCL4, also known as platelet factor 4 (PF4), in a high fat (HF) induced NASH mouse model. **Methods:** Constitutive CXCL4 knockout mice were fed HF-diet for 14 weeks. **Results:** After 14 weeks of HF treatment CXCL4 deficient animals showed a significantly increased bodyweight and elevated leptin mRNA expression levels indicating the occurrence of the metabolic syndrome compared to WT controls. Consistent with this KO mice displayed massive fatty liver degeneration with more pronounced histomorphological changes and a significantly increased hepatic triglyceride and cholesterol content. The severe NASH progression was further reflected by higher serum transaminase levels and stronger hepatic infiltration of different types of immune cells. Especially t cells, neutrophils and inflammatory macrophages were increased in livers of mice lacking CXCL4 compared to controls. Similar results could be found when analysing the immune cell infiltration in epididymal white adipose tissue (eWAT). Namely higher numbers of different t cell populations and neutrophils in CXCL4 KO mice. Those inflammatory changes finally resulted in a worsened glucose tolerance as it occurs in human obesity and type 2 diabetes. **Conclusion:** CXCL4 has a protective function in HF induced fatty liver disease since a deficiency for this chemokine resulted in faster and stronger development of diet induced NASH. Therefore PF4 provides a potential therapeutic agent for the intervention during steatohepatitis development and progression.

3.34 Different mouse models of oval cell induction can lead to reactivation of the oncofetal marker Neighbor of Punc E 11

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Background: The oncofetal marker Neighbor of Punc E 11 (Nope) is physiologically expressed on hepatoblasts in the murine fetal liver, but its expression decreases rapidly until postnatal week 3 and remains undetectable in hepatocytes of the adult liver. The aim of our study was to analyze whether expression of Nope can be reactivated in different mouse models of oval cell induction. **Methods:** We investigated C57Bl6 mice on a 3,5-diethoxycarbonyl-1,4-dihydro-collidin (DDC) diet as well as mice on a choline-deficient, ethionine-supplemented (CDE) diet in comparison with mice on normal chow. DDC and CDE diet were optionally followed by a regenerative period on normal chow. For an alternative acute liver injury, normal mice were subjected to a partial hepatectomy (PH). After defined time periods between 3 to 6 weeks, mice were sacrificed and quantitative RT-PCR and immunohistochemical costainings of the liver for Nope with CK19, A6, EpCAM (oval cell markers), E-cadherin or HNF4α (hepatocyte markers) were performed. **Results:** Partial hepatectomy did not induce any expression of Nope above background level in the adult liver. After DDC diet, the expression level of Nope was increased as measured by RT-PCR. In the CDE model, the expression level of Nope even reached a high level as in the fetal liver. Immunohistochemically, we were able to detect ductular proliferations in both injury models with coexpression of CK19, A6, EpCAM and Nope. In the DDC model followed by normal chow, the Nope expression level decreased rapidly, but small hepatocytic cells in proximity to ductular structures stained positive for Nope. Only A6 also stained positive in a minor cell fraction within periductular Nope positive hepatocytic cells. In the CDE model, we were able to detect clearly confined Nope positive hepatocytic cell clusters mainly in proximity to Nope positive ductular structures. Clearly distinguishable from these Nope expressing cell populations, expression of Nope was infrequently induced in E-cadherin and HNF4α positive parenchymal hepatocytes. **Discussion:** Reinduction of the oncofetal marker Nope in the adult liver can be effected in the DDC and in the CDE model. Nope is expressed in CK19, A6 and EpCAM positive ductular cells as well as in CK19 negative periductular small hepatocytic cells presumably representing early hepatocytes after liver injury. In conclusion, Nope is a marker for adult progenitor cells and periductular early hepatocytes in different mouse models of liver regeneration.

3.35 Transcriptional regulatory networks governing stem cell differentiation into hepatocytes in vitro

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The differentiation of embryonic stem cells (ESC) to hepatocytes offers the possibility of unlimited supply of human hepatocytes for cell therapy and biomedical research. However, current protocols achieve only a par-

tial hepatocyte differentiation. This is largely due to our poor understanding of the transcriptional regulatory networks (TRN) governing stem cell and hepatocyte plasticity. Therefore, we performed a bioinformatics approach based on whole-genome expression analysis of human stem cells, hepatocyte-like cells (HLC) and primary hepatocytes (Hep) freshly after isolation (FH) and in different culture conditions. This allowed us to identify the TRN and biological motifs associated with gain (stem cell vs. HLC) and loss of hepatocyte phenotype (FH vs. cultivated Hep). Primary human (and mouse) hepatocytes from three independent donors were cultured in collagen monolayers or sandwich systems for up to 14 days. The gene networks were identified by applying the novel CellNet algorithm and by fuzzy-c means gene clustering and gene set enrichment analysis (GSEA). The most important genes identified by these approaches were validated by real time PCR. Three different HLC models showed highly comparable phenotypes, with a partial gain of hepatocyte and concomitant fibroblast and colon gene networks. Interestingly, genes associated with failed acquisition of Hep features were largely correlated with genes repressed during primary hepatocyte cultivation. CellNet and GSEA identified novel transcription factors associated with the undesired phenotypes, including KLF5 and CDX2 for the "colon", FOXQ11 and SOX11 for the "fibroblast" and HNF1, HNF4, CAR, FXR and PXR for the Hep failed gene clusters. In both human and mouse hepatocytes, we observed thousands of upregulated genes, albeit their fold change was larger in mouse hepatocytes. These included genes involved in mRNA processing, inflammation and cell migration. The loss of mature liver phenotype and inflammation motifs were strongest in mouse hepatocytes, particularly in monolayer culture, supporting the concept that 3D matrix sustains a differentiated phenotype more effectively than 2D systems. In spite of this difference, we could detect a significant set of genes that represents an interspecies motif of alterations in primary hepatocyte culture. In conclusion, we present a blueprint for TRN involved in stem cell and hepatocyte plasticity, which will serve as basis for future interventions to improve the protocols for stem cell differentiation into hepatocytes.

3.36 Up and Down of Hedgehog Signaling leads to Down and Up of Steroidogenesis in the Liver

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The liver is one of the largest organs in our organism with multiple anabolic and catabolic functions which can be carried out simultaneously only by zonation. Recent studies showed that the morphogenic signaling pathway Hedgehog, commonly associated with embryogenesis, development processes and cancer, is active in adult hepatocytes and act as master regulator of zonation in the adult liver (Gebhardt & Matz-Soja, WJG, 2014). Our goal is the investigation of the gender dimorphism of gene regulation in the liver. Thereby we surprisingly found a regulation of steroidogenesis associated genes in hepatocytes of transgenic mice with aberrant Hedgehog signaling; yet it was assumed that steroidogenesis occurs in the liver only during embryogenesis and is down-regulated afterwards. We generated two transgenic mouse strains with an inactivated and constantly-activated Hedgehog signaling pathway, respectively. The first mouse strain has a hepatocyte-specific knockout of Smoothened (Smo), whereby the inhibition complex of the Gli transcription factors is continuously active, which results in an inactivated signaling cascade (SAC mice). The second strain has a hepatocyte-specific knockout of Patched1 (Ptch1) which lead to a permanently active Smo receptor whereby the inhibition complex of the Gli transcription factors is inactivated and the signaling cascade is continuously active (Ptch1LC1 mice). We found, that a down-regulated Hedgehog pathway results in an up-regulation of some of the steroidogenic genes and vice versa. The enzymes Star and Cyp17a1, important for the first reactions of steroidogenesis and leading to DHEA formation, are up-regulated in SAC knockout mice and decreased in expression in Ptch1LC1 knockouts in comparison to wild types. The increased levels of DHEA may explain the infertility of the homozygous female SAC mice. If Ptch1LC1 mice have an improved reproduction remains unclear yet. In contrast, the hydroxysteroid dehydrogenases (Hsd3b1/2, Hsd17b2) are down-regulated in both mouse strains. Collectively, the experiments showed a clear influence of the morphogenic Hedgehog pathway on the reproductive system and the regulation of steroidogenesis in the liver. These unexpected findings are promising for future studies to improve our understanding of the gender dimorphism of regulation in liver.

3.37 Konnex von Leber und Fettgewebe via Hedgehog Signalweg?

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Hintergrund: Der Hedgehog (Hh) Signalweg zählt, wie der Wnt/ß-Catenin Signalweg, zum einen, zu den Morphogenen und ist daher für die Embryonalentwicklung und Gewebsdifferenzierung unabdingbar. Zum anderen regulieren beide Signalkaskaden in adulten Geweben elementare, metabolische Funktionen, trotz deutlicher Aktivitätsminderung im Vergleich zur Embryogenese. Aus unseren vorangegangenen Arbeiten wissen wir, dass in der adulten Leber der Lipidstoffwechsel und die IGF-Homöostase durch den Hh Signalweg reguliert und in Balance gehalten wird. Kommt es zu Störungen der Signalkaskade in Form einer Aktivitätsminderung, entsteht ein Bild, welches der NAFLD (nicht alkoholische Fettleber) stark ähnelt. Zusätzlich konnten wir beobachten, dass die Modulation des Hh Signalweges in Hepatozyten einen drastischen Einfluss auf das Fettgewebe zu haben scheint. Diesbezüglich ist bis dato bekannt, dass dieser bei der Bildung des Fettgewebes eine zentrale Rolle einnimmt. **Methoden:** Es wurden verschiedene hepatozyten-spezifische Knockout Mausmodelle zur Aktivierung und Inaktivierung des Hh Signalweges gezüchtet. Die Aktivierung erfolgte durch Deletion von Smoothened (Smo), welches ein wichtiges Transduktorprotein in der Signalkaskade darstellt. Die Aktivierung wurde durch Inhibierung des Rezeptorproteins Patched 1 (Ptch1) erreicht. Um den Einfluss nutritiver Faktoren mit zu berücksichtigen, wurde den Tieren für 4 und 10 Wochen eine hochkalorische Diät verabreicht. Anschließend wurden die verschiedenen Fett-Spezies (braunes, subkutanes und viszerales Fettgewebe) entnommen und für weiterführende Analysen verwendet. **Ergebnisse:** Die Ergebnisse unserer Untersuchungen zeigen, dass es einen, bis dato völlig unbekannten, Zusammenhang zwischen der Aktivität des Hh Signalweges in Hepatozyten und dem Fettgewebe gibt. Dabei zeigen Mäuse mit einer hepatozytenspezifischen Inaktivierung des Hh Signalweges eine signifikante Zunahme des weißen Fettgewebes (WAT), wohingegen sich die Masse des braunen Fettgewebes (BAT) nicht ändert. Dahingegen zeigte sich, dass eine hepatozytenspezifische Aktivierung der Hh Signalkaskade zu einer deutlich geringeren Fetteinlagerung in Form von WAT, nach Gabe von HFD für 10 Wochen führt. Auf genregulatorischer Ebene können wir zeigen, dass nach Modulation des Hh Signalweges in Hepatozyten elementare, thermogenetische Gene signifikant verändert exprimiert werden. **Schlussfolgerung:** Aus den vorliegenden Ergebnissen lässt sich schlussfolgern, dass die Menge des WAT invers mit der Aktivität des Hh Signalweges in Hepatozyten korreliert. Die entscheidende Frage dabei ist, über welchen Hh-abhängigen Mechanismus die Leber mit dem Fettgewebe kommuniziert. Diese Frage zu beantworten, ist Ziel weiterführender Arbeiten.

3.38 Vitamin D Receptor Modulates Intestinal Lipid Metabolism, Adipose Tissue Inflammation and Hepatic Steatosis in Diet-induced Obese Mice

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Background: Vitamin D effects are mediated via the nuclear Vitamin D Receptor (VDR). Vitamin D insufficiency is frequently observed in obesity and NAFLD. The contribution of VDR to the pathomechanisms of these entities is, however, incompletely understood. We investigated the impact of global and intestine-specific Vitamin D signaling on the development of obesity and NAFLD in genetically-modified mice. **Methods:** Diet-induced obesity, adipose tissue (AT) inflammation and early NAFLD were analyzed in three groups of mice: Heterozygous mice served as controls. "Whole-body" Vdr-KO mice served as a model to study global effects of Vitamin D signaling. Vdr-KOhTg mice expressing an intestine-specific human VDR in the Vdr-KO background allowed studying intestinal Vdr effects. Phenotyping was performed by histological examination, gene expression analysis and measurement of serum/fecal parameters. **Results:** Vdr-KO mice were protected from HFD-induced obesity, AT inflammation and hepatic steatosis when compared to heterozygous controls. Interestingly, this protection was partly reversed in Vdr-KOhTg animals which showed increased weight gain, AT inflammation and hepatic steatosis compared to Vdr-KOs. To investigate the underlying me-

chanism, intestinal mRNA expression was analyzed with regard to genes involved in lipid metabolism. Here, changes in certain factors with known impact on peripheral LPL activity and/or intestinal lipid absorption could be detected. In line with this, changes in serum TGs and fecal fat content were observable among the different genotypes. **Conclusion:** These data point to a role of intestinal Vdr as a novel regulator of intestinal/systemic lipid metabolism modulating obesity and associated inflammatory changes in AT and liver.

3.39 α5β1 Integrins are Receptors for Bile Acids with a (Nor-)Ursodeoxycholane Scaffold

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Integrins are ubiquitously expressed cell adhesion receptors and the most prevalent bidirectional signaling molecules on the cell surface. They are involved in osmosensing in the liver [1,2,3] and facilitate the communication between the extracellular matrix and cytoplasmic proteins. Upon activation, integrins undergo large-scale conformational changes from a compact, bent state to an open, extended state [4]. A recent study combined immunofluorescence staining (IFS) experiments and molecular dynamics (MD) simulations to identify tauroursodeoxycholic acid (TUDC) as potent agonist of α5β1 integrins in hepatocytes [5]. Activation of α5β1 leads to choleresis by FAK/c-Src/MAPK dependent signaling events [6,7,8]. TUDC-induced integrin activation and subsequent signaling is sensitive to inhibition by the trihydroxylated taurocholic acid (TC), which tightly binds to α5β1 in MD simulations. However, effects of other bile acids on α5β1 integrin activation have not been investigated at the molecular level. In this study, we report on combined IFS experiments and MD simulations that indicate that α5β1 integrins are not exclusively activated by TUDC. 24-nor-ursodeoxycholic acid (norUDCA), a side chain-shortened homologue of UDCA, induces conformational changes in the βA domain of α5β1 that are similar to the one evoked by TUDC, but overall less pronounced. Conformational changes in simulations of integrin ectodomains bound with the taurine conjugate of norUDCA (TnorUDCA) and glycoursodeoxycholic acid (GUDC) were significantly less pronounced. Unconjugated UDCA, similar to the inhibitory TC, only showed insignificant alterations in the structure of the integrin ectodomain and was considered fully inactive. A ranking based on the extent of structural changes observed during the MD simulations correlates with results from IFS experiments on the efficacy of the bile acids. These results indicate that norUDCA activates α5β1 integrins and that MD simulations are able to predict different degrees of bile-acid induced integrin activation. Minor structural changes in the bile acids strongly influence their efficacy. This holds true for a comparison of TUDC (activating) vs. TC (inhibitory) and norUDCA (activating) vs. UDCA (nonactivating). We are grateful to the "Zentrum für Informations und Medientechnologie" (ZIM) at the Heinrich Heine University for computational support. This work was supported by the Deutsche Forschungsgemeinschaft through the Collaborative Research Center SFB 974 ("Communication and Systems Relevance during Liver Damage and Regeneration", Düsseldorf). **References:**
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4. Tumors, Liver Surgery and Transplantation

4.1 Die Kinase RIPK1 vermittelt eine neue molekulare Interaktion zwischen Entzündung und Zelltod in der Hepatokarzinogenese und Cholestase

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Zelltod und Entzündung sind entscheidend für die Hepatokarzinogenese. Der NF-κB-Signalweg, der durch den IKK-Komplex – bestehend aus den katalytischen Einheiten IKK1 und IKK2 sowie der regulatorischen Einheit NEMO – aktiviert wird, spielt dabei eine wichtige Rolle. So führt die Deletion von NEMO in Leberzellen (NEMO-KO) über die Aktivierung von Apoptose, kompensatorischer Regeneration und Inflammation zur spontanen Leberkrebsentstehung. Neben der Apoptose existieren weitere Zelltodsignalwege wie die Nekroptose, welche über die Kinasen RIPK1 und RIPK3 reguliert wird. Zurzeit ist nicht vollständig geklärt, wie diese Signalwege mit dem NF-κB-Signalweg interagieren und welche Rolle sie in der Karzinogenese spielen. In dieser Studie wurden Mäuse mit einem konditionellen Knockout von IKK1 und IKK2 in parenchymalen Leberzellen untersucht (IKK1/2-KO). Ähnlich wie NEMO-KO-Mäuse zeigten IKK1/2-KO-Mäuse eine Inhibierung von NF-κB sowie spontane Transaminasenerhöhungen. Im Gegensatz zu NEMO-KO-Mäusen entwickelten IKK1/2-KO-Mäuse aber keinerlei Tumore, sondern eine letale Cholestase begleitet von einer zunehmende Rarefizierung portaler Gallenwegszellen. Diese Ergebnisse deuteten an, dass IKK1 und IKK2 in der Hepatokarzinogenese und Cholestase Funktionen jenseits der NF-κB-Aktivierung haben könnten. Anschließende biochemische und genetische Experimente zeigten, dass RIPK1 den anti-karzinogenen, pro-cholestatischen Phänotyp der IKK1/2-KO-Mäuse unabhängig von RIPK3 und Nekroptose vermittelt und eine Zelltod-induzierte, kompensatorische Proliferation der parenchymalen Leberzellen inhibiert. Dementsprechend entwickelten IKK1/2/RIPK1-Triple-KO-Mäuse (nicht aber IKK1/2/RIPK3-Triple-KO-Mäuse) ein normales biliäres System, aber auch spontan Lebertumore. Massenspektrometrie und in-vitro-Kinase-Assays zeigten, dass RIPK1 ein bisher unbekanntes Phosphorylierungstarget von IKK1 und IKK2 ist und in IKK1/2-defizienten Lebern in hypophosphorylierter Form antiproliferativ wirkt. Schließlich konnte in einer Subgruppe humaner HCC-Proben eine Deletion von RIPK1 nachgewiesen werden, so dass dessen antikarzinogene Funktion auch in der humanen Leber relevant sein könnte. **Schlussfolgerung:** Wir zeigen zum ersten Mal, dass IKK1 und IKK2 neben der NF-κB-Aktivierung, die Phosphorylierung von RIPK1 vermitteln. Der IKK-abhängige Phosphorylierungsstatus von RIPK1 entscheidet über basale zelluläre Antworten auf Zelltod und kontrolliert sowohl die Hepatokarzinogenese als auch die biliäre Homöostase.

4.2 Stammzelltherapie bei ausgedehnter Leberresektion im Schwein

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Hintergrund: Nach ausgedehnter Leberresektion muss das verbleibende Restlebergewebe alle metabolischen Funktionen der Leber übernehmen. Das Operationstrauma und die hohen regenerativen Erfordernisse können zur Organdysfunktion und schließlich zum akuten Leberversagen (POLF) führen. Eine neue vielversprechende Therapieoption könnte die Transplantation von mesenchymalen Stammzellen (MSC) zur Unterstützung der Regeneration der Restleber sein. **Methoden/Ergebnisse:** In einem Großtiermodell im Schwein wurde eine 2/3 Hepatektomie mit einer warmen Ischämiezeit (150 min) durchgeführt, wodurch ein akutes postoperatives Leberversagen (POLF) ausgelöst wurde. Die Wirkung von MSC,

systemisch oder portalvenös appliziert, auf die Leberfunktion wurde evaluiert. Schweine der deutschen Landrasse wurden in 3 Gruppen (n = 4) unterteilt. Eine Gruppe bekam die Zellen zentralvenös (ZV), eine Gruppe portalvenös (PV) und eine Gruppe bekam PBS verabreicht. Die Leberfunktion wurde mittels der Plasmaverschwinde-Rate des Indocyaningrün (ICG) und dem Limon-System von PULSION über den Versuchszeitraum von 24 h gemessen. Es konnte für beide MSC Gruppen (ZV und PV) eine signifikant verbesserte Leberfunktion gegenüber der Kontrollgruppe ohne MSC gezeigt werden. Darüberhinaus verbesserten die MSC auch die leberspezifischen Funktionsparameter wie INR, Ammoniak und Serumtransaminasen. **Schlussfolgerung:** Sowohl die portalvenöse als auch die zentralvenöse Applikation von MSC führte zur signifikanten Verbesserung der Leberfunktion und klinisch relevanter hepatobiliärer Parameter nach ausgedehnter Leberresektion. Somit stellt die Stammzelltransplantation eine neue vielversprechende Therapieoption zur Vermeidung des postoperativen Leberversagens dar.

4.3 Tumor infiltrating B cells producing antitumor active immunoglobulins in resected hepatocellular carcinoma prolong patient survival

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Background: The immunological microenvironment of hepatocellular carcinoma (HCC) influences patient outcome, however, the role of B cells remains unclear. This study investigated effects of local B-cell infiltration in HCC cohorts on patient survival and immunological and molecular tumor microenvironment. **Methods:** Gene expression of 2 independent HCC tissue databases was compared using microarrays. Tissue of resected HCCs was stained for H&E, CD 20, CD 79a, CD 138 and immunoglobulins. Besides histomorphologic evaluation, the immunohistochemical stainings were analysed for the respective cell numbers separately for tumor area, infiltrative margin and distant liver stroma. These findings were correlated with clinical data and patient outcome. **Results:** Gene expression analysis of full cancer transcriptomes (N = 2158) revealed an immunological cluster in HCC tissue that mainly contained immunoglobulin fragments. More specifically, in an independent patient cohort (N = 242) that compares HCC with non tumorous liver tissue high expression of these B-cell associated genes was associated with better patient outcome (P = 0.0149). Conclusively, the immunohistochemical analysis of an independent cohort of resected HCC (N = 119) demonstrated that infiltration of HCCs by CD 20+ cells (P = 0.004) and CD 79a+ cells (P = 0.038) at the infiltrative margin were associated with prolonged patient survival. Further, the immunoglobulin fragments that were identified in the gene expression analysis were detected at high levels in patients with dense B-cell infiltration. **Conclusion:** Infiltration of HCCs by B cells is associated with prolonged patient survival. Further, a distinct B-cell like immunoglobulin profile of HCCs was identified that goes along with better patient outcome. We suggest that B cells contribute to local tumor control by secreting increased levels of immunoglobulins with antitumor activity.

4.4 A steatotic environment promotes the progression of hepatocellular carcinoma via direct and indirect mechanisms

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The metabolic syndrome and hepatic steatosis are independent risk factors for the progression of hepatocellular carcinoma (HCC). Still, the underlying mechanisms are only incompletely understood. The aim of this study was to analyze the influence of a steatotic environment on HCC cells in vitro, in a syngeneic orthotopic murine HCC model and in clinical HCC samples. **Methods and Results:** Murine Hepa129 HCC cells were implanted into the liver of C3H/HeN control mice or mice fed with a high-fat diet (HFD). Until then, the HFD had caused obesity and hyperlipidemia as well as significant hepatic steatosis but no inflammation. Two weeks after HCC cell injection, tumors grown in steatotic environment were significantly larger, revealed higher expression of matrix metalloproteases (MMPs) and invasive growth while tumors formed in normal liver tissue showed no infiltration into the surrounding liver parenchyma. Also Ki67 staining and histological analysis of 129 human HCC samples revealed a higher proliferation index in HCCs grown in steatotic livers compared to liver tissues without significant lipid accumulation in the surrounding liver tissue. In line with this, in vitro stimulation of HCC cells with conditioned medium (CM) of steatotic primary human hepatocytes (PHH) caused a significant increase of proliferation compared to CM from control PHHs. Interestingly, not only non-tumorous liver tissue but also HCCs grown in mice fed high-fat diet revealed higher triglyceride levels compared to HCCs in control livers. Accordingly, analysis of the triglyceride content in HCC and corresponding non-tumorous liver tissue specimens of 18 patients showed a significant correlation. To simulate hyperlipidemia in vitro free fatty acids were added to the cell culture medium of HCC cells, which caused a dose dependent induction of cellular lipid accumulation accompanied by enhanced migratory activity and MMP expression. Furthermore, mRNA and immunohistochemical analysis revealed higher alpha-smooth muscle actin (alpha-sma) expression in the stroma of tumors grown in steatotic livers. Alpha-sma is a marker of activated hepatic stellate cells (HSC)/myofibroblasts, which are known to promote the tumorigenicity of HCC cells. Fitting to this, incubation with CM of steatotic HCC cells significantly induced HSC activation and proliferation of already activated HSC, respectively, compared to CM of control HCC cells. **Conclusions:** A steatotic environment appears to promote HCC progression via different mechanisms. Steatotic hepatocytes secrete factors inducing HCC growth. Moreover, lipid accumulation in the tumor cells themselves induces invasive growth and a desmoplastic reaction.

4.5 Accumulation of hepatitis B surface antigen promotes the development of alpha-1 antitrypsin mutation-related liver disease

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Introduction: α1-antitrypsin (AAT) constitutes the major serum protease inhibitor that is produced predominantly in hepatocytes. An AAT mutation termed PiZ leads to its polymerization and retention in the endoplasmic reticulum (ER) thereby causing the development of AAT deficiency, the third most common lethal genetic condition in humans. While up to 40% of PiZZ patients develop liver cirrhosis, the phenotype of the disease is highly variable and the factors contributing its development remain unknown. Aims & methodology: To mimic the consequences of a hepatitis B (HBV) infection-related ER stress, we crossbred PiZ mice with animals overexpressing the large hepatitis B surface protein (HBs mice). Livers were evaluated by quantitative RT-PCR, immunoblotting, histological/immunological staining and biochemical assays. **Results:** HBs-PiZ mice were viable and developed normally. At two months of age, the double transgenic mice displayed a significantly stronger liver injury (ALT: PiZ-HBs 88, HBs 53, PiZ 32, $p < 0.05$ for both) and a distinct CHOP overexpression. Although both AAT and HBs is retained in the hepatocyte ER, the proteins display a distinct, non-overlapping accumulation pattern. Compared to single transgenic animals, 10 months old PiZ-HBs mice harbour a more pronounced liver fibrosis. At 14 months of age, double transgenic animals demonstrated significantly increased liver-body weight ratio (HBs-AAT 0.08, HBs 0.07, PiZ 0.05, $p < 0.05$ for both) higher tumor load (Double 141, HBs 36, PiZ 3.7 mm2, $p < 0.05$ for both) and larger tumor nodules. **Conclusions:** Our results suggest that accumulation of HBs protein accelerates the development of PiZ-related liver disease.

4.6 Aggressive systemic mastocytosis of the liver with cholangitis

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Introduction: Mastocytosis is a clonal, neoplastic mast cell proliferation. It may be divided into cutaneous and systemic mastocytosis. In cutaneous mastocytosis, the course of disease is often indolent. If other organs with or without skin lesions are involved, WHO-criteria for systemic mastocytosis are fulfilled. If organ function is additionally impaired (C-findings) in systemic mastocytosis, an aggressive course of disease can be assumed. Sclerosing cholangitis is a chronic disease of bile ducts in or outside the liver leading to inflammation of bile ducts with obstructive fibrosis and consecutive development of liver fibrosis and cirrhosis. The underlying cause of the disease is unknown. There is an association with inflammatory bowel disease. **Case presentation:** Here we present a case of a 26 year old male patient with aggressive systemic mastocytosis with involvement of the liver mimicking primary sclerosing cholangitis. The patient presented in emergency department in reduced general condition complaining about progredient weakness, fatigue, and vomiting. He reported on a previous diagnosis of ulcerative colitis, primary sclerosing cholangitis, systemic mastocytosis and liver cirrhosis in an external hospital. Routine laboratory diagnostic test showed cholestasis with elevated levels of alkaline phosphatase and mildly elevated gamma glutamyl transferase levels. Further laboratory tests gave no indication for other underlying diseases, such as viral infection, autoimmune disease or drug-induced/toxic liver injury. Furthermore, gastroscopy and colonoscopy was done. Pathological examination revealed multifocal, dense clusters of more than 15 large, in part spindle shaped cells immunoreactive with antibodies against the mast cell markers CD117/c-KIT and tryptase. Taken together diagnosis of systemic mastocytosis was established and the previous diagnosis of primary sclerosing cholangitis was critically questioned. During follow-up, iliac crest biopsy was performed, showing bone marrow infiltrates of mast cells with aberrant immune phenotype with coexpression of CD25, CD117, and tryptase with c-KIT point mutation KIT D816V. In addition, liver biopsy was done showing infiltrates of clustered atypical mast cells with positivity for CD117 and tryptase in the portal tracts, acinar liver parenchyma, bile ducts and vessels within fibrotic liver tissue. Cytokeratin 7 immunohistochemistry demonstrated significant degenerative changes of the original bile ducts, ductular proliferations, and ductular metaplasia of periportal hepatocytes indicating chronic cholestasis. No signs of specific cholangiopathies were found. In summary, aggressive systemic mastocytosis was diagnosed. **Conclusion:** Aggressive systemic mastocytosis is a rare cause of liver injury and may therefore be misdiagnosed. Systemic mastocytosis affecting the liver may involve bile ducts in a fashion typical for other causes of cholangitis, i.e. primary sclerosing cholangitis.

4.7 Analysis of transcriptomic changes during cold ischaemia in time-zero biopsies of liver allografts

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Liver transplantation remains the only treatment option for many patients with end-stage liver disease or acute liver failure. Despite substatial progress in preventing graft failure over the past deacades, a substatial number of patents still suffer from progressive graft dysfunction, subsequently leading to re-transplantation or death. Thus identifying the moleculare causes of graft failure would certainly be a major step ahead in organ preservation and patient survival. Cold ischemia time appears to be a good predictor among the factors influencing patient and graft survival. Meta-analysis have recently suggested increasing graft failures in patients receiving organs with cold ischemia time of more than 8 – 9 hours. Thus, identifying the molecular changes in grafts with more than 8 hours of cold ischemia may eventually aid to better preserve organs and help to further decrease graft failure. In order to identify early molecular changes in liver grafts associated with subsequent graft failure we collected time zero biopsies after liver reperfusion from 93 consecutive patients. Extensive transcriptomic profiling was performed using the gene expression microarrays. Supervised analysis of molecular changes associated with increasing cold ischemia time included major networks associated with major changes in Inflammation, cell death and lipid metabolism. IL1, IL8, and IL32 were identified as key molecules associated with increased cold ischemia time. All three molecules showed an average increase throughout the time course of increasing cold ischemia. No overlap was seen compared to murine models of ischemia/reperfusion injury. In conclusion transcriptomic profiling of time zero biopsies in patients with liver transplantation demonstrated enrichment of Inflammation, cell death and lipid metabolism with Il1, IL8, and IL32 being key members of the associated networks. A better understanding of their role during cold ischemia may aid better organ preservation strategies in the future.

4.8 Expression of genes and pathways associated with colorectal liver metastases in an orthotopic and syngeneic mouse model

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Background: Colorectal cancer (CRC) is the second most common cause of cancer-related death in men and women. Systemic disease with metastatic spread to distant sites remains a major challenge. The aim of this study was to investigate the molecular requirements necessary during the evolution of CRC cells in the liver environment. **Methods:** Cells from the CMT-93 cell line (originating from mouse CRC) were cultured in RPMI. For analysis in vivo, one million cells were injected via the portal vein into syngeneic mice livers, leading to the formation of stable metastases within three weeks. RNA of the CMT-93 cells and liver metastases as well as from matched normal liver was utilised to evaluate expression profiles of more than 20,000 genes through RNA-Seq analysis. **Results:** A total of 8,046 genes were differentially expressed when CMT-93 cells propagated in liver: 4,872 genes were up-regulated and 3,174 down-regulated. Comparing the cultured CRC cells with their liver metastases, gene expression of VEGF, STAT1, IFG1, and CXCL12 was identified as highly up-regulated, whereas vimentin, PLAUR, CCND1, and CDKN2A were down-regulated. These genes are highly relevant on crucial pathways with regard to differentiation, proliferation, and metabolic processes as determined during GO term analyses also performed. **Discussion:** Bioinformatic analysis of these RNA-Seq data may help reveal biological processes on different pathways involved during metastasis development in the liver environment. In the present study, a novel differential gene expression pattern was identified and further studies will be necessary to investigate new targets in the treatment of CRC liver metastasis.

4.9 Association of immune cell infiltration and tumor suppression in hepatocellular carcinoma

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Background: Different inflammatory states, e.g. chronic hepatitis B or C virus infection, have been shown to contribute to the development of hepatocellular carcinoma (HCC). However, the molecular mechanisms and regulation of immune cell infiltration during hepatocarcinogenesis are still poorly understood. Recently, we applied an integrative genomic and transcriptomic approach in HCC which revealed that loss of chromosome 8p is associated with poor prognosis. In addition, we showed that the two chromosome 8p genes SORBS3 and SH2D4A are functional tumor suppressor genes in vitro and in vivo. In this study, we aimed at linking SORBS3 and SH2D4A expression with tumor-infiltrating immune

cells. **Methods:** Tumor-infiltrating T cells (CD3, CD4, CD8, FOXP3), B cells (CD20), macrophages (CD68) and granulocytes (NASDCL) were immunohistochemically determined on tissue microarrays including 127 HCC specimens with comprehensive pathological and clinical data including overall patient survival. Additionally, SORBS3 and SH2D4A expression were assessed on the same tissue microarray. Prognostic impact was evaluated using the Kaplan-Meier method and Cox regression model. **Results:** In our cohort of 127 HCC patients including cryptogenic, alcohol-related and viral HCCs, high numbers of CD20-positive tumor-infiltrating B cells were associated with better prognosis based on Kaplan-Meier survival analysis ($p < 0.001$). In contract, tumor-infiltrating T cells, macrophages and granulocytes were not associated with patient outcome. Consistent with functional data showing that SORBS3 acts as a tumor suppressor gene, we found that patients with SORBS3-negative HCC had a higher risk to die compared to the population with SORBS3-positive tumor cells (Hazard ratio (HR)= 1.89, 95% CI: 1.07 – 4.16, $p < 0.05$). In contrast, SH2D4A was positively correlated with the presence of CD3, CD4, CD8 and FOXP3-positive tumor infiltrating immune cells (Pearson $p < 0.05$). Thereby, the association with CD8 and FOXP3 was strongest suggesting a potential role of SH2D4A in T cell infiltration. **Conclusion:** In this study, we found that CD20-positive B cells and the tumor suppressor gene SORBS3 are associated with patient outcome, whereas, SH2D4A is associated with T cell infiltration. Thus, both chromosome 8p tumor suppressor genes may be potential modulators of tumor infiltrating immune cells preventing the immune system from destroying HCC tumor cells.

4.10 Autophagy impairment and ERK and p38 activation are central mediators of irinotecan-induced steatohepatitis

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Treatment with irinotecan is associated with the development of chemotherapy-associated steatohepatitis (CASH), which significantly increases the risk of perioperative morbidity and mortality. The aim of this study was to unravel the molecular mechanisms of this phenomenon. **Methods:** Mechanisms of irinotecan induced steatohepatitis were studied in primary human hepatocytes in vitro, irinotecan treated mice and liver specimens from irinotecan-treated patients. **Results:** Irinotecan dose-dependently induced accumulation of free fatty acids and triglycerides, formation of reactive oxygen species (ROS), pro-inflammatory gene expression as well as activation of MAPK ERK and p38 in hepatocytes. Moreover, irinotecan treatment significantly inhibited ATG7 and VSP34 expression accompanied by lower LC3II/I ratio and increased p62 protein levels indicative of reduced autophagy. ROS-scavengers and ERK-inhibition almost completely abrogated irinotecan-induced inflammatory gene expression but had only a slight effect on lipid accumulation. However, p38 inhibition diminished irinotecan effects on autophagy and steatosis. Also in mice irinotecan treatment (50 mg/kg) induced significant hepatic steatosis and inflammation accompanied by MAPK-activation and reduced authophagy. This was confirmed in livers samples of irinotecan-treated patients, which revealed a significant induction of ERK- and p38-activation and a marked reduction of markers of autophagy in comparison to normal liver tissue. Of note, pretreatment with multi-tyrosine kinase inhibitor sorafenib inhibited the irinotecan induced inflammatory response in hepatocytes in vitro as well as in irinotecan treated mice. **Conclusion:** ERK and p38 activation are critical mediators of irinotecan-induced steatohepatitis. (Pre)treatment with sorafenib appears as therapeutic option for prevention of CASH.

4.11 CUX1 in liver cancer: experimental study in hypoxia model

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Background: 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. It favors the expression of oncogenes and survival factors, especially in stress condition, thus supporting tumorigenesis. Here, we show CUX1 activity during hypoxia in liver cancer cells. **Materials/Methods:** CUX1was knocked down and its targets were analysed by RT-qPCR in HepG2 and Hep3B cells under hypoxic and/or normal culture condition. The hypoxia condition was established by 24 h treatment with 150 µM CoCl2 or with 0.5% O2 atmosphere. **Results:** Hypoxia determined the up-regulation of HIF1-alpha (Hypoxia inducible factor1-alpha) and a stable or up-regulated expression of its inhibitor FIH-1 (SLC2A1) up to 24 h prolonged hypoxia. VEGFA was significantly overexpressed. Knockdown of CUX1 determined a significant down-regulation of HIF-1alpha, FIH-1 and VEGFA. Interestingly, the expression of CDKN1A was only attenuated after CUX1 knock down and hypoxic stress. **Conclusions:** CUX1 exerts an oncogenic role in liver cancer by sustaining the survival mechanism beyond hypoxia. CUX1 silencing results in suppression of the hypoxia inducible factor and its target VEGFA causing a block of cell cycle in liver cancer cells modulated by the stable expression of CDKN1A.

4.12 Deregulation of the Hippo/YAP pathway drives chromosomal instability (CIN) in hepatocellular carcinoma by regulating the transcription factor FoxM1

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The Hippo/YAP signaling pathway is an important regulator of cell proliferation and organ growth. YAP has been described as an oncogene, promoting hepatomegaly and the formation of hepatocellular carcinoma (HCC) (Zender et al. 2006). However, the precise downstream mechanisms of YAP-dependent tumorigenesis are only poorly understood. In this study we examined the role of YAP in the regulation of chromosomal instability (CIN) in hepatocarcinogenesis. Using siRNA treatments and Luciferase assays we showed that YAP regulates the transcription factor FoxM1 on a transcriptional level in different HCC cell lines. Moreover, we observed an interaction between YAP and FoxM1. Importantly, YAP was able to regulate a signature consisting of 25 genes, which characterizes tumors with chromosomal instability (CIN signature, Carter et al., 2006). Induction of this signature was FoxM1 dependent as illustrated by siRNA perturbation experiments and treatment with a FoxM1 inhibitor. In vivo, liver-specific overexpression of an inducible and constitutively active YAP isoform led to massive liver overgrowth and eventually cancer formation (Tschaharganeh et al. 2013). These mice also showed increased expression levels of FoxM1 and CIN signature genes and exhibited signs of chromosomal instability such as nuclei polymorphisms, pathological mitosis, increased number of centrosomes and positive pH2AX staining, which is a marker of DNA double strand breaks. To show that YAP expression correlates with FoxM1 and CIN genes in human tissues, a tissue microarray containing 115 HCCs was stained using immunohistochemistry. A significant correlation between YAP enrichment and expression of FoxM1 and CIN signature genes was observed. To investigate the impact of the YAP-dependent CIN signature on patient survival, gene expression data from 242 HCC patients were used (Roessler et al. 2010). Patients

with high expression level of the CIN signature showed the worst overall survival and an early cancer recurrence. We also identified the most important genes for patient subclustering. A score based on the expression level of these four identified genes (TTK, TPX2, MAD2L1, MCM2) significantly correlated with chromosomal gains and losses of HCC patients, indicating that these genes alone suffice to identify patients with CIN. These data demonstrate that YAP and its downstream effector (and binding partner) FoxM1 represent master regulators of a CIN signature, which characterizes HCC patients with poor clinical outcome. Four CIN genes could be used as biomarkers to identify patients that express the CIN signature and might benefit from treatment with YAP and/or FoxM1 inhibitors. **References:** [1] Carter SL, et al., Nat Genet. 2006;38(9):1043 – 8. [2] Roessler S, et al., Cancer Res. 2010;70(24):10202 – 12. [3] Tschaharganeh D, et al., Gastroenterol. 2013 144(7):1530 – 1542 [4] Zender L, et al., Cell 2006;125 (7):1253 – 1267.

4.13 Different oncogenic potential in the mouse liver of mutant forms of the p110alpha catalytic subunit of phosphoinositide-3-kinase (PIK3CA)

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Background: PIK3CA, encoding the catalytic subunit p110α of class I phosphoinositide-3-kinase (PI3K), is mutated or overexpressed in many tumors, including hepatocellular carcinoma (HCC), where it is supposed to act as an oncogene. Here, we developed new mouse models to investigate the oncogenic effects of gain-of-function PIK3CA mutations and identified downstream PI3K signaling components in the mouse liver. **Methods:** PIK3CA mutants G1633A:E545K and A1340G:H1047R were overexpressed in the liver of 129/Sv-C57BL/6 mice via hydrodynamic gene delivery (HGD). Three, six, nine and twelve months after HGD, preneoplastic lesions and liver tumors were analyzed and compared histomorphologically. Putative PIK3CA targets were examined via Western Blot analysis and immunhistochemistry. In addition, microarray analysis was performed in order to identify novel targets of PIK3CA in HCC. **Results:** Three and six months after HGD, the overexpression of different PIK3CA mutant forms in 129/Sv-C57BL/6 mice resulted in the development of multiple liver preneoplastic lesions and liver tumors. However, overexpression of PIK3CA-E545K exhibited a much more pronounced oncogenic potential than PIK3CA-H1047R. Indeed, all mice injected with PIK3CA-E545K developed liver tumors by six months post HGD, whereas only 10%, 10%, and 30% of mice injected with PIK3CA-H1047R developed liver tumors by six, nine, and twelve months after HGD, respectively. Furthermore, overexpression of key enzymes of lipid metabolism and proteins involved in cell growth or survival were identified as downstream targets of oncogenic PIK3CA in mice. **Conclusion:** Our investigation indicates that PIK3CA mutant forms, G1633A:E545K and A1340G:H1047R, are oncogenic in the mouse liver albeit with a different oncogenic potential. In addition, we identified a number of PIK3CA targets in the liver, whose investigation might be helpful both for a better understanding of the molecular pathogenesis of HCC and the development of new therapeutic strategies against this deadly disease.

4.14 Early ultrastructural hepatocellular alterations after hydrodynamic tail vein injection

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Background: Hydrodynamic tail vein injection (hydrodynamic transfection, HT) is one of the latest murine gene transfer models in liver cancer research. Transfected DNA constructs are composed of two plasmids, one containing the gene of interest and the other the sleeping Beauty-transposase allowing a stable integration of this gene into the hepatocyte genome. Rapid tail vein injection of a large volume of DNA-solution induces an acute cardiac congestion, resulting in a reflux into the liver, mainly in acinus zone 3. It has been shown that HT induces this hydrodynamic force leading to permeabilization of the fenestred sinusoidal endothelium. Nevertheless, mechanisms of plasmid incorporation into hepatocytes remain unclear. **Methods:** In this study, 2 ml volume of empty vector or saline solution (control) were hydrodynamically injected into the tail vain of anaesthesized C57BL/6J/129Sv mice. Liver tissue was resected at different time points (1, 2, 5, 10, 20, 30 or 60 minutes after HT) and quickly fixed with buffered 1% osmium tetroxide. For electron microscopic evaluation, ultrathin sections were cut from glycidether embedded blocks. **Results:** After 1 minute, hepatocytes near the central venule in acinus zone 3 reveal some small membran bound vesicles in the cytoplasm. After few more minutes, vesicles increased in size to vacuoles and in number. Vacuoles can often be found in proximity to the nucleus. They are optically empty and contain no electron dense material. Some but other hepatocytes revealed signs of cell damage, i.e. swollen mitochondria, dilated endoplasmatic reticulum and golgi apparatus and disrupted plasma membranes, whereas most hepatocytes appeared normal. All findings were similar after the injection of the empty vector and the saline solution as well. **Discussion and conclusion:** Our ultramorphological findings indicate that hydrodynamic plasmid transfer to the hepatocytes is accomplished via active or passive endocytosis with an entrapment of the plasmid in multiple membran bound vesicles and vacuoles. Hepatocytes containing vacuoles did not show disrupted plasma membranes or other signs of cell damage, therefore mechanisms like i.e. membrane poration are unlikely, but cannot be entirely excluded. The vesicle formation is a nonspecfic process, independent from the plasmid itself or its enclosed components. It remains to be clarified which active or passive mechanisms are involved in vesicle generation and finally how the DNA enters the hepatocellular nucleus.

4.15 Enhanced expression of c-myc in hepatocytes promotes initiation and progression of alcoholic liver disease

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Background & Aims: Alcohol exposure may result in the overexpression of certain oncogenes in human cells thereby increasing the intracellular concentration of reactive oxygen species (ROS) and, thus, triggering initiation and progression of alcoholic liver disease (ALD). We previously showed that prolonged c-myc expression in hepatocytes leads to spontaneous fibrogenesis and end-stage high-latency tumor development. In the present study, we hypothesized that c-myc overexpression might exert a crucial role in the development of ALD. **Methods:** Expression of c-myc was measured in biopsies of patients with ALD by quantitative real-time PCR (qPCR) and by immunohistochemistry (IHC). ALD in mice carrying transgenic over-expression of c-myc in hepatocytes (alb-myctg) and wildtype (WT) controls was induced by administration of ethanol (EtOH) containing Lieber DeCarli diet for 4 weeks. Primary hepatocytes were isolated from WT and alb-myctg mice, subjected to EtOH treatment and investigated for markers of cell cycle progression and oxidative stress by Western blotting, immunofluorescence and electron microscopy. **Results:** Hepatic c-myc was strongly up-regulated in human patients with advanced ALD and in WT mice fed with EtOH-diet. Conversely, the overexpression of c-myc in hepatocytes led to early ballooning degeneration, increase of liver collagen deposition and ethanol-induced hepatic lipotoxicity, in conjunction with excessive CYP2E1-derived ROS after EtOH-diet. Unexpectedly, alb-myctg-livers displayed impaired cell proliferation resulting in remarkable hepatic hypertrophy and hepatocyte enlargement in response to EtOH challenge. Moreover, EtOH-fed alb-myctg mice exhibited profound dramatic changes in the mitochondrial morphology associated with mitochondrial dysfunction. Consistently, alb-myctg-derived primary hepatocytes showed blockade of proliferation and dramatic increase of cellular ROS, in response to EtOH challenge. Mechanistically, elevated c-myc expression and ethanol uptake synergistically lead to strong AKT activation, Mdm2 phosphorylation and as a consequence to inhibition of p53. **Conclusions:** Our findings show that the proto-oncogen c-myc accelerated the progression of ALD thereby increasing collagen deposition and intracellular concentrations of oxidants through a p53-dependent mechanism. These results render c-myc as a plausible novel diagnostic and prognostic marker for early detection of ALD.

4.16 Isolation of primary human hepatocytes from human liver tissue after portal vein embolization

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Primary human hepatocytes are an important resource for basic research, pharmaceutical testing, and therapeutic concepts in regenerative medicine. Primary human hepatocytes can be isolated from resected liver tissue. Preoperative portal vein embolization (PVE) is increasingly used to decrease the risk of delayed postoperative liver regeneration by induction of selective hypertrophy of the future remnant liver tissue. We here investigate the effect of PVE on the outcome of hepatocyte isolation. Primary human hepatocytes were isolated from liver tissue obtained from partial hepatectomies (n = 190) using the two-step collagenase perfusion technique followed by Percoll purification. Of these hepatectomies, 27 isolations (14.2%) were performed using liver tissue obtained from patients undergoing PVE prior to surgery. All isolations were characterized using parameters established to be relevant for the outcome of hepatocyte isolation. The isolation outcomes of the PVE and the non-PVE groups were then compared before and after Percoll purification. Metabolic parameters (transaminases, urea, albumin, and vascular endothelial growth factor secretion) were measured in the supernatant of cultured hepatocytes over a period of 6 days (PVE: n = 4, non-PVE: n = 3). The PVE and non-PVE groups were similar in regard to donor parameters (sex, age, indication for surgery), isolation parameters (liver weight, cold ischemic time), and the quality of the liver tissue. The mean initial viable cell yield did not differ between the PVE and non-PVE groups. The initial viability was slightly better in the PVE-group (77.8 ± 2.03% vs. 74.4 ± 1.06%). The mean viable cell yield (p = 0.819) and the mean viability (p = 0.141) after Percoll purification did not differ between the groups. PVE had no effect on enzyme leakage and metabolic activity of cultured hepatocytes. PVE does not negatively affect the outcome of primary human hepatocyte isolation. Liver tissue after PVE is a suitable source for the isolation of primary human hepatocytes and is equivalent to untreated liver tissue in regard to cell yield and viability.

4.17 Hepatic B cell leukemia-3 attenuates chemically-induced hepatocarcinogenesis in mice

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Background: The transcriptional NFkappaB-coactivator B cell leukemia-3 (Bcl-3) is a molecular regulator of cell death and proliferation and highly expressed in the liver. Bcl-3, when dysregulated, has been shown to be widely expressed in several cancer types including hepatocellular carcinoma (HCC), but its precise function in pathogenesis is still unknown. **Methods:** To investigate the role of hepatic Bcl-3 during initiation and progression of HCC, 7 – 10 day-old, male mice exhibiting an increased hepatocyte-specific expression of Bcl-3 (alfp-Cre:bcl-3, Bcl-3Hep) and wild type (wt) littermates received a single intraperitoneal injection of 25 µg diethylnitrosamine (DEN) followed by continuous treatment with 0.5 g/l phenobarbital (PB) dissolved in drinking water. Blood and liver tissue were harvested at 40 weeks of age for further analysis. **Results:** Remarkably, Bcl-3Hep mice exhibited less and smaller tumour nodules in comparison to wt controls in response to DEN/PB. Reduced HCC formation was accompanied by decreased hepatocyte death and reduced amounts of compensatory proliferation as Ki-67 labeling and qRT-PCR analyses of p53, cMyc and mTOR revealed. In agreement, the phosphorylation of c-Jun N-terminal kinase (JNK) and extracellular-signal regulated kinase (ERK) was diminished in the tumour and tumour surrounding tissue of Bcl-3Hep mice, while a higher degree of activated p38 and NFkappaB p65, p50 and p52 was detectable. Compared to the wt, the absolute number of intrahepatic macrophages, CD8+ T cells and B cells was reduced in DEN/PB-treated Bcl-3Hep mice, and B cells exhibited a decreased expression of the activation markers CD81 and BLNK. **Conclusion:** Hepatic Bcl-3 exerts beneficial effects during hepatocarcinogenesis through regulation of cell death, compensatory proliferation and inflammatory processes.

4.18 Heterologous, costimulation-assisted vaccinations drive potent cellular immune responses to cancer

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Immunotherapy of cancer has emerged as an effective means for the treatment of solid cancer. The therapeutic efficacy of current therapies, however, is hampered by the low immunogenicity of the currently available vaccines that typically induce immune responses at or below the threshold of detection. Bacterial or viral vectors are able to overcome this limitation but require laborious vector design and are thus unsuitable for individually tailored vaccination approaches. We sought to develop a novel cancer vaccination that induces potent immune responses while preserving a maximum of flexibility with regard to the antigen of choice. Since co-stimulatory antibodies are potent stimulators of T cell expansion, we tested various combinations of agonistic costimulatory antibodies in combination with peptides and TLR agonists. We observed optimal CD8 T cell expansion when peptide vaccination was given in combination with the TLR3 agonist Poly I:C and CD40 agonistic antibodies. To enable short-term amplification of the cancer-specific immune response, we combined this costimulation-assisted vaccination with various primary immunizations. Surprisingly, the combination of the costimulation-based vaccination with a dendritic cell immunization led to massive amplification of CD8 T cell immune responses targeting either neoantigens or tumor-associated antigens. In therapeutic setting, the novel vaccination led to complete regression of subcutaneous tumor after vaccination against a single, high affinity antigen. Our results demonstrate that optimized prime-boost vaccinations are able to strongly amplify cancer-specific immune responses thus warranting their further evaluation for the immunotherapy of cancer patients.

4.19 High levels of the soluble programmed death-ligand (sPD-L1) identify hepatocellular carcinoma patients with a poor prognosis

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Aim: Immunotherapy in cancer is a recent and very promising approach, namely the inhibition of the PD/PD-L1 axis. New treatment strategies in HCC are desperately wanted. Here we aimed to investigate the prognostic value of a soluble form of PD-L1 in hepatocellular carcinoma patients. **Methods:** HCC patients were prospectively recruited and sPD-L1 (serum soluble programmed death-ligand 1) levels were determined. sPD-L1 levels were compared to stages of cirrhosis and HCC stages. The association of the sPD-L1 levels and overall survival (OS) was assessed in uni- and multivariate Cox regression models. **Results:** 215 patients with HCC were included. Mean duration of follow up was 298 ± 304 days with a range of 1 – 1464 days. 19 (8.8%) patients underwent liver transplantation and 60 (27.9%) patients died within the observation time. The median serum sPD-L1 concentration in patients with HCC was 0.5 ng/ml (range 0.03 – 6.04, mean 0.73 ± 0.82). Soluble PD-L1 levels positively correlated with the stage of liver cirrhosis as well with stages of HCC. sPD-L1 furthermore correlated positively with a marker of macrophage activation (sCD163) and inflamma-tion (CRP). According to sPD-L1 levels in a healthy control cohort we defined a cut-off for high-level sPD-L1 (> 0.8 ng/ml). Patients with high serum sPD-L1 concentrations had an increased mortality risk (hazard ratio (HR) 2.513, 95% confidence interval (CI) 1.497 – 4.220, P < 0.001). Furthermore, high sPD-L1 levels were associated with mortali-ty independently from BCLC, the ALBI grade and a AFP level > 400 ng/ml in a multivariate Cox regression model. **Conclusions:** We conclude that a high sPD-L1 level is a possible prognostic indicator for a poor outcome in hepatocellular carcinoma patients. The predictive value of sPD-L1 levels for a successful anti PD1/PD-L1 therapy should be investigated in the future.

4.20 Identifikation neuer differenziell regulierter Mediatoren im Rahmen der Leberregeneration

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Einleitung: Die Leber ist das größte Stoffwechselorgan des Menschen. Neben der Metabolisierung von Endo- und Xenobiotika findet hier auch die Synthese von Plasmaproteine sowie die Bildung der Galle statt. Darüber hinaus spielt die Leber eine wichtige Rolle bei der angeborenen und adaptiven Immunabwehr. Infolge von Schädigungen des Leberparenchyms setzt ein multifaktorieller Prozess ein, welcher durch eine kompensatorische Hyperplasie des verbleibenden gesunden Gewebes zur Regeneration der Leber führt. Obwohl diverse Untersuchungen zur Regulation der Regeneration durchgeführt wurden, konnte diese bisher nicht vollständig geklärt werden. **Methoden:** Zur Identifizierung weiterer möglicher relevanter Faktoren für die Regeneration wurden zeitaufgelöste differenziell regulierte Faktoren nach 2/3 PHx an Mäusen identifiziert. Hierzu wurden zu jedem Zeitpunkt Kontrollmäuse (Sham) generiert. Untersucht wurde die RNA- und Protein-Expression diverser Zytokine, Wachstumsfaktoren und Akut-Phase-Proteine mittels Chip-Analyse bzw. real-time PCR und Multiplex-Analyse im Gesamt-Leberlysat bzw. im Serum. **Ergebnis:** Für die Zytokine IL-6, IL-10, IL-33, CCL-2, CCL-9 und den Wachstumsfaktor Nrg-1 wurde eine differenzielle Regulation der Expression detektiert. Die Expression der Akut-Phase-Proteine α-2-Makroglobulin, Hepcidin, SAP, SAA2, SAA3 und ORM1 variierte ebenfalls deutlich. **Schlussfolgerung:** Während bereits Funktionen für Hepcidin, IL-6 und IL-10 während der Regeneration beschrieben wurden, ist die Relevanz der übrigen Faktoren für die Leberregeneration bisher ungeklärt. Die Faktoren CCL2, CCL9, IL-33 und Nrg1 wurden im Zusammenhang mit Hepatektomien bisher noch nicht beschrieben.

4.21 Identifizierung angiogener Faktoren nach Applikation von Hep3B mit mesenchymalen Stammzellen in der immundefizienten Maus

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Hintergrund: Die Therapie des hepatozelluläre Karzinoms (HCC), weltweit die fünfthäufigste Tumorart, erfordert zumeist die umfangreiche Teilleberresektion. Folglich ist ein hohes regeneratives Potential der Restleber erforderlich. Die pro-proliferativen Eigenschaften von mesenchymalen Stammzellen (MSC) könnten die Regeneration bestmöglich unterstützen. Allerdings ist die Frage nach der Stimulation des Tumorwachstums durch MSC nach wie vor ungeklärt. Das Ziel der Arbeit war es daher, den Einfluss von MSC auf das Tumorwachstum zu untersuchen und insbesondere evtl. beteiligte Zytokine zu identifizieren. **Methoden:** Ektope epitheliale Tumore wurden in vivo durch die subcutane Injektion von HEP3B, die Co-Injektion mit MSC bzw. die zeitabhängige Gabe von MSC (i.v.; 3 d und 8 d nach Hep3B-Injektion) in der immundefizienten Pfp/Rag2-/- Maus induziert. 15 Tag nach der Injektion wurde das Gewicht der Tumore bestimmt und ein Teil des Tumorgewebes für die Analyse von Zytokinen und angiogenen Faktoren mittels Proteome Profiler (R&D Systems) verwendet. **Ergebnisse:** Nach Co-Injektion von Hep3B und MSC wurde ein signifikant höheres Tumorgewicht im Vergleich zur Injektion von Hep3B alleine (401,7 g ± 148 vs. 118,9 g ± 44) nachgewiesen. Die zweimalige MSC-Injektion 3 d und 8 d nach der Hep3B-Injektion führte zu deutlich kleineren Tumoren (42,9 g ± 15,8). Im Vergleich zur Co-Injektion wurden in den alleinigen Hep3B-Tumoren signifikant höhere bFGF- und IL-32-Spiegel gemessen. In den kleineren Tumoren nach sukzessiver MSC-Gabe war im Vergleich zu den beiden anderen Gruppen eine signifikant verringerte Expression von Angiogenin, ICAM-1, IL-8, IL-32 und PDGF-AA messbar. Gleichzeitig war die Expression von INF-γ erhöht. Die sukzessive Gabe der MSC führte auch zur signifikant verringerten Expression der proinflammatorischen Zytokine IL-6 und Lipocalin. Da unter keinen Umständen MSC in den Tumoren gefunden wurden, muss man davon ausgehen, dass diese Veränderungen vom Tumor selbst ausgingen. **Schlussfolgerung:** Während die gleichzeitige Gabe von MSC und Tumorzellen das Tumorwachstum begünstigte, war dieses durch die sukzessive Gabe der Hep3B und der MSC vermindert. Die Veränderungen im Zytokinprofil des Tumors könnten dafür verantwortlich sein.

4.22 Inactivation of fatty acid synthase impairs hepatocarcinogenesis driven by AKT in mice

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Background: Cumulating evidence underlines the crucial role of aberrant lipid biosyntesis in human hepatocellular carcinoma (HCC). Here, we investigated the oncogenic potential of fatty acid synthase (FASN), the master regulator of de novo lipogenesis, in the mouse liver. **Methods:** FASN was overexpressed in the mouse liver, either alone or in combination with activated N-Ras, c-Met, or SCD1, via hydrodynamic injection. Activated AKT was overexpressed via hydrodynamic injection in livers of conditional FASN or Rictor knockout mice. FASN was suppressed in human hepatoma cell lines via a chemical inhibitor and specific small interfering RNA. **Results:** Overexpression of FASN, either alone or in combination with other genes associated with hepatocarcinogenesis, did not induce histological liver alterations. In contrast, genetic ablation of FASN resulted in the complete inhibition of hepatocarcinogenesis in AKT-overexpressing mice. In human HCC cell lines, FASN inactivation led to a decline in cell proliferation and a rise in apoptosis, which were paralleled by a decrease in the levels of phosphorylated/activated AKT, an event controlled by the mammalian target of rapamycin complex 2 (mTORC2). Downregulation of AKT phosphorylation/activation following FASN inactivation was associated with strong inhibition of rapamycin-insensitive companion of mTOR (Rictor), the major component of mTORC2, at a post-transcriptional level. Finally, genetic ablation of Rictor impaired AKT-driven hepatocarcinogenesis in mice. **Conclusions:** FASN is not oncogenic per se in the mouse liver, but is necessary for AKT-driven hepatocarcinogenesis. Pharmacological blockade of FASN might be highly useful in the treatment of human HCC characterized by activation of the AKT pathway.

4.23 Induction of stemness features and activation of prognostically adverse genomic alterations in hepatocellular carcinoma by field cancerization

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Background: HCC is a paradigm for inflammatory cancers. A pre-existing chronic liver damage creates an adverse microenvironment that promotes tumor-initiation and progression. The cancer stem cell origin provides a plausible explanation for the resulting phenotypic heterogeneity of hepatocellular cancers. However, despite profound therapeutic implications the prognostic relevance of CSCs and their cellular localization within the tumor formation remain controversial. **Methods:** Expression levels and localization of established CSC markers were assessed in preneoplastic lesions and two independent cohorts of HCC patients using qRT-PCR and imunohistochemistry. Integrative whole genome and transcriptome analyses of different tumor regions as well as tumor-surrounding liver (SL) were performed to identify associated molecular alterations and integrated with our existing HCC database. **Results:** While classical HCC markers were induced in tumor tissue, activation of CSCs was predominantly observed in SL and continuously decreased from preneoplastic lesions to HCC. Consistently, genomic and transcriptomic profiles of the different regions were quite distinct. Progressive increase in genetic alterations and activation of pathways related to proliferation

was observed in the tumor tissue. However, while the SL showed enrichment of stemness pathways, the invasive tumor margin (TM) was characterized by inflammatory and EMT-related gene sets. Consistently, integration of the different signatures with our database of 53 HCC revealed that the TM is a critical determinant of the patient outcome. **Conclusion:** The CSC phenotype of HCC might be induced by a pro-oncogenic field effect that develops in the permissive liver microenvironment. Activation of key oncogenic features as well as immune-response signaling indicates that the cross-talk between tumor and microenvironment might be a promising therapeutic and/or preventive target.

4.24 Ginkgo biloba differentially affects untransformed and malignant cells in the liver

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Ginkgo biloba (EGb) is a widely used botanical drug with diverse biological properties. Several reports indicate that EGb confers both preventive effects as well as anti-tumorigenic properties in a variety of tumors, including hepatocellular carcinoma (HCC). We here evaluate the functional and mechanistic effects of EGb on human hepatocellular carcinoma cells as well as untransformed hepatocytes. Human hepatoma cell lines, primary human HCC cells and immortalized human hepatocytes were exposed to various concentrations (0 – 1000 ug/ml) of EGb 761, a well-defined and quantified EGb leaf extract. Effects on proliferation, apoptosis and oxidative stress were evaluated after 72 h of EGb exposure. Molecular changes were assessed by gene expression microrarrys, qRT-PCR, Western Blotting and confocal microscopy. EGb administration significantly impaired proliferation and induced apoptosis in hepatoma cells as well as hepatocytes. However, median IC 50 for the hepatoma cells was dramatically lower than in hepatocytes suggesting a differential response of EGb on normal and malignant cells. Consistently, while EGb induced a significant reduction in both colony and sphere forming ability as well as reactive oxygen species (ROS) generation in hepatoma cells, the treatment caused no mentionable changes in untransformed cells. Mechanistically, anti-tumorigenic properties of EGb were exerted via inhibition of genes associated with cell cycle (e.g. CCND 1, MAPK8), survival (e.g. AKT, MEK) and impaired redox potential (e.g. KEAP1). EGb differentially affects hepatocytes and human hepatoma cells. While anti-tumorigenic and pro-apoptotic changes were induced in hepatoma cells, untransformed cells remained unaffected suggesting that EGb could be safely used for both preventive as well as therapeutic strategies. Future work will explore the molecular mechanism responsible for the observed differential response in normal and cancer cells.

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

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Background: MicroRNAs (miRNAs) are small non-coding RNAs that modulate the activity of gene expression. Only few data describe the role and expression of miRNAs in liver ischemia-reperfusion (IR) injury. IR is a major cause of liver damage during surgical procedures including liver transplantation. Therefore, in this study we sought to identify the potential involvement of miRNAs in IR injury in mice. **Method:** PCR-based miRNA microarray was performed with the liver samples of mice subjected to IR injury and sham. The effect of miR-1224 was assessed by serum enzyme analysis and histological examination of liver. The levels of pro-inflammatory cytokines were determined by quantitative real-time PCR. BRDU and TUNEL stains assessed the functions of miRNA and its potential target. **Results:** Among the top differentially regulated miRNAs, miR-1224 was found to be highly upregulated in both liver tissue and serum of IR mice, which positively correlated with liver damage markers. Similarl, upregulation of miR-1224 was observed in both H2O2 – induced and TNFα – stimulated hepatic AML 12 and Hepa 1 – 6 cells. Furthermore, miR- 1224 overexpression resulted in a decrease of cell proliferation, a decreased activation of AKT/ERK proteins and induction of caspase 3/8 -mediated apoptosis. Reporter assay confirmed that miR-1224 directly targeted the 3'UTR of the transcription factor Sp1. Sp1 knockdown recapitulated the miR-1224-induced phenotype. Finally, the upregulation of miR-1224 and downregulation of Sp1 were observed in both liver and serum of human acute liver failure samples. **Conclusion:** Liver injury due to IR and H2O2 induces miR-1224 in both liver and serum and promotes apoptosis via inhibition of its direct target gene Sp1.

4.26 Molecular imaging of Cyclin E1 represents an indicator of acute and chronic liver disease

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Background: Acute and chronic liver diseases are frequently associated with proliferation of Hepatocytes and Hepatic stellate cells (HSC). The proliferation of immune cells in this context is poorly investigated. Sustained activation of these processes can lead to fibrosis, cirrhosis and hepatocellular carcinoma (HCC). E-cyclins (E1, E2) control transition into S-phase of the cell cycle and play a crucial role during carcinogenesis. Our own preliminary work demonstrated an essential role of Cyclin E1 for liver fibrogenesis and HCC development in mice. Thus Cyclin E1 could be a suitable diagnostic marker for indication of liver fibrosis and HCC. The aim of the present study was to develop novel strategies for early diagnosis of aberrant Cyclin E1 expression and identification of novel Cyclin E1 expressing cell populations in liver fibrosis and HCC. **Methods:** We generated a reporter system enabling the non-invasive detection of Cyclin E1 gene expression in murine cell lines (Hepa1 – 6) and in transgenic mice (C57BL/6). In our approach, the naive murine Cyclin E1 promoter controls the expression of fluorescence reporters suitable for intravital microscopy, non-invasive imaging and tracking of Cyclin E1 expressing cells *in situ* Reporter-based tracking of Cyclin E1 expression was investigated in mouse models of ectopic and orthotopic allograft transplantation, Diethylnitrosamine (DEN)-induced hepatocarcinogenesis and CCl_4-mediated acute liver injury. **Results:** *In vivo*, non-invasive imaging of Cyclin E1 expression could be successfully performed in whole animals using tumor models of allograft transplantations and DEN-induced hepatocarcinogenesis. These experiments revealed that Cyclin E1 expression was more pronounced during liver tumor initiation and less important for tumor progression. *In situ*, distribution patterns of Cyclin E1 expression in tumors and tissue could be efficiently determined at macro- and microscopically levels. Beyond, the reporter system revealed a high sensitivity for tracking and identifying Cyclin E1 expressing cells, for instance during metastasis formation. Analysis of the hepatic tumor environment identified for the first time Cyclin E1 expressing non-parenchymal cells, which in part could be assigned to a CD 11b positive cell population. Cyclin E1 expression in CD 11b positive cells could also be detected in acute liver injury, suggesting that the role of Cyclin E1 and proliferation of immune cells has been underestimated so far. **Conclusion:** The generated reporter system is an effective tool for identifying and tracking of Cyclin E1 expression in whole-body, organs and most cell populations. This allows re-defining the relevance of cell proliferation for homeostasis and disease; moreover we will be able to better define windows for therapeutic interventions.

4.27 Processing of MIA2 is important for its tumor suppressor function in hepatocellular carcinoma

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Melanoma Inhibitory Activity 2 (MIA2) is a 451 amino acid protein exclusively expressed by hepatocytes in the liver. In hepatocellular carcinoma (HCC), however, MIA2 expression is strongly downregulated and acts as a tumor suppressor. In contrast to the functional role of MIA2, the biochemical properties were not elucidated until today. The aim of this study was to determine modifications and processing of MIA2 and their

relevance to MIA2 function as a tumor suppressor. **Methods and Results:** Computer algorithm analysis suggested membrane localization of MIA2 which was confirmed by cell fractioning and by labeling of membranous proteins. Interestingly, MIA2 was found to be processed resulting in a soluble, extracellular MIA2 representing the N-terminal part. This processing was highly important for MIA2 function on HCC cells because stable (re-)expression of MIA2 in HCC cells significantly impaired proliferation, however, a specific HCC cell clone re-expressing MIA2 but lacking the processing capability did not show reduction of proliferation. Next, we determined the cleavage site of MIA2 by site-directed mutagenesis. Amino acid exchange within this site resulted in changes of MIA2 processing depending on the respective amino acid exchange. A deletion of two amino acids at this site even completely abolished MIA2 processing. To determine the enzyme involved in MIA2 cleavage, a Fluorescence Resonance Energy Transfer (FRET) assay was performed. Here, a peptide containing the cleavage site with its surrounding amino acid sequence was synthesized, using FAM as the fluorophore linked to the N-terminus and Dabcyl as a Quencher at the C-terminal end of the peptide. Subsequent analysis revealed that the enzyme directly binds to the characterized amino acid sequence and cleaves within. First inhibitor studies revealed that the enzyme is Ca2+-dependent and is most probably a serine or cysteine protease. Possible candidate enzymes will be further analyzed using the FRET assay as well as siRNA technology. **Conclusion:** Processing of MIA2 is important for its tumor suppressor function in HCC cells. A deeper understanding of this process including the identification of the protease responsible for MIA2 cleavage may provide novel diagnostic markers and therapeutic targets for this aggressive tumor.

4.28 Role of new pathways in liver regeneration after acute and chronic liver damage

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The liver is a vital organ that performs many biological functions (e.g. synthesis of bile and blood proteins, detoxification, glycogen storage, innate immunity). Interestingly, the liver also has a unique ability to regenerate following the loss of liver mass. Loss of at least 30% of liver mass, leads to synchronized proliferation of mature hepatocytes and rapid restoration of liver mass via compensatory hyperplasia. When a liver has recovered after chronic damage caused by e.g. viral infection the whole complex architecture of the liver must be restored. Hepatic stellate cells (HSCs) are mainly known for their contribution to fibrogenesis in chronic liver diseases, but their role and function in liver regeneration remains unclear. In summary, both processes are orchestrated by distinct signaling cascades involving components of the innate immune system, cytokines, bile acids and growth factors. For this reason, our studies aim to investigate the role of different signaling pathways and factors that are essential in both, acute and chronic liver damage. To analyze the dynamic processes during liver regeneration, we performed two common mouse models: PHx (acute liver damage) and BDL (chronic liver damage) in different Knockout-mice with deficiencies in innate immune responses. Using kinetics we examined the gene expression profile of liver tissue after PHx and BDL. H/E-staining of liver sections and cytokine ELISAs were performed and serum protein levels and bile acid concentrations were analyzed. Taken together, we have found new factors that may be essential for liver regeneration after acute and chronic liver damage, however, their precise role in liver regeneration has to be fully elucidated.

4.29 Role of the bile acid receptor TGR5 (Gpbar-1) in gastrointestinal tumors

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Background: The G-protein coupled bile acid receptor TGR5 (GPBAR1) is expressed in biliary epithelial cells of the liver as well as epithelial cell of the intestine. Receptor activation induces proliferation and anti-apoptotic effects in liver but in the intestine its function is relatively unclear. It was shown that secondary bile acids (BA) are involved in the development of different gastrointestinal (GI) malignant tumors of the intestine and liver. High TGR5 expression levels were detected in cell lines generated from adenocarcinomas of the esophagus and cholangiocarcinoma (CCA). Interestingly, high concentrations of secondary BAs (DCA and LCA) were found in faeces of colon cancer patients. Since these two secondary BAs represent potent TGR5 ligands, aim of this project is to uncover the role of TGR5 in GI tumor formation with a main focus on colon cancer and CCA. **Methods:** Immunofluorescence staining for TGR5 were performed of human colorectal cancer (CRC) and healthy tissues. TGR5 mRNA expression was measured by quantitative realtime PCR. Human CCA cell lines (TFK-1, EGI-1) and CRC cell lines (Caco2, HT29, LoVo) were stimulated with different BAs (TLC, DC, TDCA) and a TGR5 agonist and proliferation was determined by BrdU incorporation. Following stimulation with BA or TGR5 agonists phosphorylation of the epidermal growth factor receptor (EGFR) or ERK1/2 in CCA cell lines were investigated by western blot analysis. TGR5 knockdown in TFK-1 cells was achieved by siRNA transfection. **Results:** TGR5 mRNA levels in human CRC tissue were higher as compared to controls. Immunofluorescence staining demonstrated TGR5 localization in CD163- positive macrophages of healthy colon samples and tumor cells of human CRC samples with higher intensity in tumor cells than in normal intestinal epithelial cells. Human CRC cell lines Caco2 and HT29, expressing high TGR5 levels, proliferate to a higher extent when stimulated with TDCA than the LoVo cells with low TGR5 expression. TGR5 stimulation resulted in significantly increased EGFR and ERK1/2 phosphorylation in human CCA cell lines as well as significantly increased proliferation which is abolished in TFK-1 cells, transfected with TGR5 siRNA compared to controls. **Discussion/Conclusion:** TGR5 mRNA levels and RVLR2 positive stainings were found to be increased in human CRC tissue as compared to control tissue. An overexpression of TGR5 was also found in human CCA tissue. Activation of TGR5 in CRC and CCA cell lines resulted in increased cell proliferation and phosphorylation of both the EGFR and ERK1/2. Knockdown of TGR5 in the CCA cell line TFK-1 abrogated the proliferative response induced by TGR5 ligands. These data suggest a role for the BA receptor TGR5 in the proliferative response of CCA and CRC tumor cells. To investigate the in vivo relevance of these findings a 8 – 10 month DCA feeding mouse model is in progress to characterize the development of colonic adenomas and carcinomas in dependence to the TGR5 genotype.

4.30 Salvage in situ liver transection (ISLT) for patients with unresectable liver tumors and insufficient volume increase of the future liver remnant after portal vein embolization

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Introduction: In about one third of patients, portal vein embolization (PVE) fails to increase the future liver remnant (FLR) prior to extended hepatectomy, leaving these patients non-resectable. The newly established in situ liver transection (ISLT or ALPPS) procedure for extended hepatectomy, that combines portal vein ligation and parenchymal transection prior to second stage resection, proved to induce rapid volume increase of the FLR. The feasibility of ISLT as salvage procedure after failed PVE has been explored. **Patients & Methods:** 17 ISLT procedures in non-cirrhotic livers were performed and analyzed, including 4 salvage ISLT subsequently after insufficient volume gain following PVE. ISLT was indicated when FLR/body weight (BW) ratio was below 0.5. Follow up CT-scan for FLR volume evaluation was routinely performed on postoperative day 3, to determine date of stage two operation. Patient characteristics, volume increase, postoperative complications and outcomes were analyzed. **Results:** The FLR volume after ISLT exceeded in all patients the critical FLR/BW ratio of 0.5 and subsequent R0 resection could be achieved. The mean FLR volume gain was 60% (± 23%) on day 3 post ISLT. Even after failed PVE, salvage ISLT achieved a mean volume gain of 62% (± 15%) and the mean FLR increased by 229 ml (± 68 ml) in these four patients. Mean time to second stage operation after ISLT was 7 days (range 4 – 14 days). 13 patients (76%) experienced post operative complications grade III and higher according to Clavien-Dindo classification. Median and 1y/2y overall survival were 6.3 months and 47.1%/35.3% respectively. **Conclusion:** ISLT has become an established surgical technique, which allows curative resection of initially unresectable liver tumors. It is an effective and reliable alternative to PVE. Isolated transection of the liver after failed PVE (salvage ISLT or ALPPS) represents a very

helpful procedure, leading to similar results as primary ISLT in theses otherwise non-resectable patients.

4.31 Selective targeting of tumor-associated macrophages in hepatocellular carcinoma

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Hepatocellular carcinoma (HCC) is the third most frequent cause of cancer-related mortality worldwide showing a high resistance to therapy, sorafenib being the only drug prolonging life of patients for a few months. Tumor-associated macrophages (TAMs), which show alternative (M2) polarization and suppress immune function and promote tumor growth, angiogenesis and metastasis are valid therapeutic targets within the tumor microenvironment. Therefore, selective targeting of M2 macrophages in the tumors is a highly promising anti-tumor strategy. However, this has not yet been achieved. Recently, the peptide M2pep was described to bind selectively to murine M2 macrophages. Aim of this project is to investigate if M2pep can be used to target TAMs in HCCs. By flow cytometric analysis and confocal laser scanning microscopy we showed selective binding of the peptide to M2-differentiated macrophages isolated from murine bone marrow. Furthermore, by coupling the pro-apoptotic KLA-peptide to M2pep, we could show killing of bone marrow-derived M2 macrophages. In the next steps, we will investigate the anti-HCC therapeutic potential of M2pep-KLA in tumor spheroids incorporated with M1 or M2 polarized macrophages, a 3D cellular cancer model that is of relevance as an increasingly preferred model to investigate the therapeutic potential of new treatment approaches. Hence, the treatment of HCC-spheroids, co-cultivated with macrophages, with the pro-apoptotic fusion peptide should cause the depletion of the macrophages and therefore should lead to the suppression of the tumor promoting functions of TAMs, including migration, proliferation or angiogenesis.

4.32 Study of the relationship between explant histopathology and hepatocellular carcinoma post living donor liver transplantation

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Hepatocellular carcinoma (HCC) is the fifth most common malignant tumors worldwide. Orthotopic liver transplantation (OLT) is the only therapeutic option to treat both the cancer and the underlying liver disease. **Methods:** The study was conducted on 32 patients of HCC who underwent LDLT,. The explanted liver was sectioned & examined großly & microscopically for the degree of differentiation of tumour& microvascular invasion if present (prognostic histologic indicators). Follow up of patients was done including α-feto-protein, Ultrasound, Triphasic CT and other imaging when needed. **Results:** The patients' age ranged from 15 to 68 years. Three patients were diagnosed by explant histopathology as non HCC lesions and excluded. Six of the remaining 29 patients (20.7%) experienced HCC recurrence (1 hepatic, 4 extrahepatic " 40% in the lung" and 1 both hepatic & extrahepatic). All patients with recurrence had history of preoperative TACE. No significant relationship were found between the recurrence with either preoperative Child classification or AFP level (P = 0.41 & 0.12 respectively). 34.5%, 17.2%, 27.6%, 17.2% and 3.4% of patients had 1, 2, 3 and 4 focal lesions (P = 0.18). The mean values of diameter of largest focal lesion in patients with and without recurrence were 4.08 ± 1.5 and 2.80 ± 1.2 respectively (P = 0.05). Regarding the size: 31.0%, 44.8%, 17.2%, and 6.9% of patients had tumour size of 1 – 3 cm, 3 – 6 cm, 6 – 9 cm and > 9 cm respectively that is significantly related to HCC recurrence (P = 0.029) and the mean values of patients with and without recurrence were 7.88 ± 3.145 and 4.28 ± 1.9 respectively (P = 0.009). By explant histopathology 79.3% of patients were well differentiated and 20.7% were moderately differentiated insignificantly related to recurrence. Vascular invasion occurred in 6.89% of patients that is insignificantly related to HCC recurrence (P = 0.34). Patients with higher AFP preop. Larger tumour size or preoperative TACE showed a higher rate of HCC recurrence.

4.33 The value of intraoperative White-Test for biliary leakage following hepatic resection

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Background: Bile leakage testing may help to detect and reduce the incidence of biliary leakage after hepatic resection. A meta-analysis of medium quality studies (individual cohort studies including low quality randomized controlled trials) revealed, that the use of the White-Test leads to a significant reduction of post-operative biliary leakage (OR: 0.3 (95% CI: 0.14, 0.63), p = 0.002). **Patients and Methods:** Retrospective analysis of patients undergoing liver resection in 2012 and 2013. Patients were split into two groups (+/- intraoperative White-Test). Biliary leakage was defined in accordance with ISGLS definition for biliary leakage. **Results:** A total of 251 consecutive patients undergoing liver resection were included in the analysis. Data are given as median with interquartile range. Age was 64 years (51; 71). 135 patients (54%) underwent major resection. White-Test was carried out in 69 (27%) vs. 182 (73%) without any specific intraoperative leakage-test. A total of 62 bile leakages occurred post-operatively (25%, grade A:16, grade B:39, grade C:7). There was no statistically significant difference in patients with White-Test (13/69:18.8%) compared to no intraoperative testing (49/182:26.9%) (p = 0.18; OR:0.63). In patients with major resections, the trend for less biliary leakages was stronger when using White-Test (10/48:20.8% vs. 28/87; 32.2%) but still not statistically significant (p = 0.14; OR: 0.55). **Conclusion:** Our findings do not confirm former findings for the use of the White-Test to identify bile leaks intraoperatively. There is a trend towards better outcome when using the White-Test. Nonetheless, there is a requirement for a RCT with adequately powered sample-size and clear definitions of outcome parameters to identify the clinical value of the test.

4.34 Wisp1 as an early stage marker of human hepatocellular carcinoma (HCC)?

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Background and aims: Microarray analysis of TGF-β treated mouse hepatocytes revealed Wisp1 as the most regulated gene. As TGF-β is upregulated during HCC development and progression, we analyzed Wisp1 expression and function in HCC. **Methods:** Wisp1 mRNA expression was analyzed in 7 publically available HCC patient cohorts. Wisp1 protein expression was determined in patients' serum and liver tissue (46 paired and tissue microarray (TMA) with 220 independent samples) and correlated with clinical parameters and survival. Functional evaluation was performed after Wisp1 overexpression and TGF-β treatment using HuH7 cells. **Results:** In different HCC cohorts, Wisp1 was downregulated as compared to surrounding tissue but slightly upregulated as compared to normal liver. TMA analysis revealed correlation of Wisp1 with gender, moderate and well differentiation and early stages of HCC. By trend, high Wisp1 indicated better survival of patients especially in early stages. In HCC patients, serum contained, in trend, higher Wisp1 levels as compared to healthy and cirrhotic patients. Wisp1 expression was mainly located in well-differentiated cancer cells, while in surrounding tissue Wisp1 was predominantly found in hepatocytes associated with inflammation. In HuH7 cells, TGF-β1 induced Wisp1 expression transiently at early time points (4 h, 8 h). Further, Wisp1 overexpression inhibited Smad2 phosphorylation and CAGA reporter activity after TGF-β1 stimulation indicating a negative regulation loop of Wisp1 for the TGF-β pathway. No significant impact of Wisp1 overexpression on cell viability, apoptosis, adhesion ability and sensitivity to Sorafenib was detected. **Conclusions:** Taken together, our data suggest Wisp1 as a marker for early HCC providing a negative feedback for TGF-β signaling.

5. Viral Hepatitis and Immunology

5.1 Adaptive transfer of human T cells redirected against HBV results in reduced viral loads and induced immune responses in humanized mice

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Effective T cell responses are essential to resolve HBV infection. However, in chronic hepatitis B patients HBV-specific T cell responses appear severely impaired. Adoptive transfer of T cells engineered to express an HBV-specific T cell receptor (TCR) may contribute to viral load reduction and reconstitution of HBV-specific immune responses. Aim of the study was to assess the engraftment capabilities and antiviral activity of HBV-specific redirected human effector T cells in vivo using HBV infected human liver chimeric uPA/SCID/ILγR2 (USG) mice. **Methods:** PMBCs isolated from healthy human blood were cultured for 1 week to activate and enrich the T cell population (+IL2 600 IU/ml; CD3 0.05 µg/ml). TCR-HBVs183 – 91 mRNA (restricted on MHC class I HLA-A201) was amplified by in vitro transcription and transduced via electroporation into activated T cells before being injected in uninfected or HBV-infected, haplotype matched (HLA-A201) or mismatched (HLA-A101) humanized mice. Virological markers and gene expression changes were determined by qRT-PCR and immunofluorescence. **Results:** One single injection of TCR transduced T cells in HLA-matched HBV infected mice already provoked a temporary reduction of viremia (median Δ0.5 log after 4 days), while multiple T cell injections (3 times in 12 days) resulted in progressive viremia reduction (median Δ1 log) exclusively in HLA-matched humanized mice, since viremia remained stable in similarly treated mismatched HBV-infected mice. Intrahepatic levels of HBV transcripts appeared 50% lower compared to controls and an increase of apoptosis markers (Caspase3) was detected in mice receiving TCR transduced cells, but not in controls. Also ALT levels and expression of human inflammatory cytokines and markers (e.g. IFNgamma, TNFalpha, IL-10, hTGFbeta, caspase8, granzyme B) were clearly enhanced in HBV-infected mice that received haplotype matched HBV-specific T cells, but not in mismatched controls. The successful reconstitution of mouse livers with human immune cells was also confirmed by the increased amounts of hCD8+ T cells (from day 6 to 12) detected by immunofluorescence in mouse livers. **Conclusions:** This pilot study aiming at exploring the potential of adoptive T cell therapy in chronic HBV infection using human liver chimeric mice indicates that repeated injection of TCR transduced human CD8+ T cells can induce a clear immune-mediated reduction of serological and intrahepatic viral loads without occurrence, at least for the time frame of investigation, of unspecific immune responses or graft versus host reactions.

5.2 Induction of innate and adaptive immune responses after stopping NA therapy in HBeAg negative chronic hepatitis B

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Stopping nucleos(t)ide analogue (NA) treatment before HBsAg loss is currently debated as a concept to induce HBsAg decline. In a prospective single center study we show that stopping NA treatment in HBeAg-negative patients is safe and leads to a significant decline of HBsAg. To understand mechanisms leading to HBsAg decline, the three HBV surface proteins (S-, L and MHBs), core-related antigen (HBcrAg), cytokine/chemokine levels and HBV-specific T cell responses were measured before and after stop of NA therapy. In 13/15 patients a virological relapse with HBV-DNA and ALT flairs at week 4 (n = 4), 8 (n = 11), 24 (n = 1) and week 36 (n = 1) was detected. On the last observation two patients lost HBsAg, with one having anti-HBs. One additional patient achieved HBsAg of 0.2 IU/ml. The peak of HBV-DNA and HBcrAg during relapse but not ALT correlated strongly with fold decline of HBsAg at week 48. Overall levels of S-, L- and MHBs significantly declined as seen for qHBsAg. Plasma cytokines/chemokine levels of IL-10, IL-12 and TNF-α were significantly increased early (4 weeks) and CXCL10 (IP-10) at week 8 after treatment stop. Interestingly, some but not all patients showed increased functional HBV-specific CD4 and CD8 T cell responses after stop of NA treatment. In a prospective trial, we showed that stopping NA therapy leads to a significant HBsAg decline strongest in patients with highest HBV-DNA and HBcrAg levels. Our data suggests that the induction of cytotoxic and non-cytotoxic innate and adaptive immune responses during relapse are important for HBsAg decline.

5.3 Charakterisierung des NK-Zellpools bei HIV/HCV-koinfizierten Patienten

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Hintergrund: Bei HIV/HCV-koinfizierten findet sich im Vergleich zu HCV-monoinfizierten Patienten ein rascheres Fortschreiten der Lebererkrankung. Natürliche Killer-(NK)-Zellen spielen eine wichtige Rolle bei der Anti-HCV-Immunantwort und können die Progression einer Leberfibrose beeinflussen. Da sowohl die HIV- als auch die HCV-Infektion mit spezifischen Veränderungen des NK-Zellpools assoziiert sind, untersuchten wir in der vorliegenden Arbeit Funktion und Phänotyp von NK-Zellen bei HIV/HCV-Koinfizierten. **Methoden:** NK-Zellen von 19 HIV/HCV-koinfizierten, 31 HIV-monoinfizierten, 39 HCV-monoinfizierten und 40 gesunden Personen wurden durchflusszytometrisch hinsichtlich Phänotyp und Funktion untersucht. **Ergebnisse:** Die NK-Zellexpression der Differenzierungs-/Reifungsmarker CD27/57/62L/127 unterschied sich nicht zwischen HIV(+)/HCV(+) und HIV(+)/HCV(-) Patienten. Im Vergleich zu den gesunden Kontrollen und HIV(-)/HCV(+) Patienten fand sich bei den HIV(+)/HCV(-) bzw. HIV(+)/HCV(+) jedoch eine signifikant niedrigere Expression von CD27, CD62L und CD127 bei gleichzeitig erhöhter Expression von CD57. In Bezug auf die untersuchten NK-Zellrezeptoren (NKG2A/C/D, NKp30/46) fanden sich nur bei NKp30 signifikante Unterschiede zwischen HIV(+) und HIV(+)/HCV(+) Patienten, wobei NK-Zellen von Koinfizierten die niedrigste Expression aufwiesen. Dagegen fand sich in beiden HIV-infizierten Patientengruppen eine signifikant höhere Expression von NKG2C bei gleichzeitig niedrigerer Expression von NKG2A als bei den gesunden Kontrollen und den HCV-monoinfizierten Patienten. In funktionellen Untersuchungen zeigte sich, dass NK-Zellen koinfizierter Personen eine im Vergleich zu allen anderen Kontrollgruppen signifikant eingeschränkte Degranulation aufwiesen, während sich bei HIV(-)/HCV(+) die niedrigste IFN-γ-Produktion fand. **Zusammenfassung:** Unsere Untersuchungen deuten darauf hin, dass die HIV-Infektion bei HIV(+)/HCV(+) Patienten einen dominanten Einfluss auf den NK Zell-Pool hat. Angesichts der Bedeutung von NK-Zellen für den HCV-Infektionsverlauf könnte dies eine mögliche Ursache des aggravierten klinischen Verlaufs einer HIV/HCV-Koinfektion darstellen.

5.4 Deficiency of the B Cell-Activating Factor Receptor Results in Limited CD169+ Macrophage Function during Viral Infection

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The B cell-activating factor (BAFF) is critical for B cell development and humoral immunity in mice and humans. While the role of BAFF in B cells has been widely described, its role in innate immunity remains unknown. Using BAFF receptor (BAFFR)-deficient mice, we characterized BAFFR-related innate and adaptive immune functions following infection with vesicular stomatitis virus (VSV) and lymphocytic choriomeningitis virus (LCMV). We identified a critical role for BAFFR signaling in the generation and maintenance of the CD169+ macrophage compartment.

Consequently, Baffr-/- mice exhibited limited induction of innate type I interferon production after viral infection. Lack of BAFFR signaling reduced virus amplification and presentation following viral infection, resulting in highly reduced antiviral adaptive immune responses. As a consequence, BAFFR-deficient mice showed exacerbated and fatal disease after viral infection. Mechanistically, transient lack of B cells in Baffr-/- animals resulted in limited lymphotoxin expression, which is critical for maintenance of CD169+ cells. In conclusion, BAFFR signaling affects both innate and adaptive immune activation during viral infections.

5.5 Amphipathic, nucleic acid-based polymers optimized to treat hepatitis B virus infection in patients do not harbour immune stimulatory properties in primary isolated blood or liver cells

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Background: Nucleic acid polymers (NAPs) are phosphorothioate oligonucleotides which interfere protein interactions involved in viral replication. This property is length dependent and sequence-independent. It has been demonstrated that NAPs are active against hepatitis B virus (HBV) where the antiviral mechanism is still being investigated. In this study, the immune stimulatory properties of NAPs optimized for the treatment of HBV infection in vivo and in the clinic were examined in primary isolated human blood and liver cells. **Methods:** Human peripheral blood mononuclear cells (PBMCs) as well as primary isolated hepatocytes and Kupffer cells were treated with different concentrations of NAPs. REP 2006 is a 40mer degenerate NAP (dN)40 previously shown to have residual pro-inflammatory activity due to CpG content and REP 2055 is 40mer NAP containing a sequence optimized to be devoid of CpG content (dAdC)20 that retains antiviral activity and which has been shown to be effective in vivo and clinically against HBV infection. To indicate immune responsiveness, toll-like receptor ligands (polyI:C, ssRNA, ODN2216) were used as immune stimulatory controls. Total RNA was isolated and quantitative RT-PCR was performed to analyse gene expression of IFNA4, IFNB1, IFNG, IFNL2, TNF, IL6, and IL10. The intracellular uptake of CY3-labelled NAPs was visualized using fluorescence microscopy. **Results:** REP 2006 induced an inflammatory cytokine response in PBMCs comparable to the ODN2216 control. However, while ODN2216 strongly induced the expression of interferons (IFNA4, IFNB1, IFNG, IFNL2), REP 2006 did not. Here just a weak signal for IFNB1 expression could be detected when the highest concentration of 5 µM was used. The treatment with REP 2055 resulted in only a weak induction of IL6 expression in PBMC. In primary isolated human hepatocytes neither ODN2216 nor REP 2006 or REP 2055 induced the expression of any of these genes. In contrast, poly I:C-treated hepatocytes showed significant induction of IL6, TNF, IFNB1, IFNG and IFNL2. Interestingly, in Kupffer cells REP 2006 and REP 2055 induced a slight, dose-dependent expression of TNFA, whereas expression of IL6 and IL10 was not altered. A strong expression of interferon genes (IFNB1, IFNG, IFNL2) could only be induced by poly I:C. However, a weak expression signal of IFNA4 and IFNL2 was detected in KC stimulated with ODN and REP 2006. **Conclusion:** Although some synthetic nucleic acids have been shown to be able to stimulate an innate immune response in vivo and in vitro, the data presented here demonstrate that NAPs optimized to treat hepatitis B virus infection in patients do not induce antiviral responses in primary isolated blood or liver cells. We therefore hypothesize that the antiviral activity of NAPs cannot be explained by direct induction of innate antiviral responses.

5.6 Poly I:C-stimulated human non-parenchymal liver cells are potent suppressors of hepatitis C virus replication

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Background & Aims: The role of non-parenchymal liver cells as part of the hepatic, innate immune system in the defense against hepatotropic viruses such as hepatitis C is not well understood. Therefore, the aim of the study was to characterize the toll-like receptor signaling and antiviral capacity in primary human non-parenchymal liver cells. **Methods:** Primary human Kupffer cells, liver sinusoidal endothelial cells and hepatic stellate cells were isolated from liver tissue obtained after tumor resections or liver transplantations. Cells were stimulated with toll-like receptor agonists for 6 h-24 h, expression of selected cytokines was analyzed by quantitative PCR and ELISA. Furthermore, the hepatoma cell line, harboring subgenomic hepatitis C virus replicon, was co-cultured with supernatants from toll-like receptor-stimulated cells. **Results:** Non-parenchymal liver cells expressed inflammatory cytokines and trigged activation of down-stream signaling pathways in response to toll-like receptor stimulation in a cell-type specific manner. Only supernatants of poly I:C-activated cells mediated an antiviral activity against hepatitis C virus, assessed in the con1 replicon system. This antiviral effect was abolished by blocking the interferon alpha receptor 2 using neutralizing antibodies. However no neutralization could be achieved by blocking IFN-α and/or IFN-β. Furthermore, liver sinusoidal endothelial cells isolated from hepatitis C virus-positive donors showed higher responsiveness to poly I:C, indicated by significantly elevated expression of IFN-β and IFN-λ compared to uninfected controls. **Conclusions:** Non-parenchymal liver cells respond to most of the toll-like receptor ligands by producing inflammatory cytokines. Toll-like receptor-induced antiviral effects in these cells, are restricted to poly I:C stimulation and seems to be mediated by type-I interferons. These findings shed new light on the relevance of non-parenchymal liver cells in the pathogenesis of hepatitis C virus.

5.7 Characterization and functional modulation of HBV-specific CD4+ T cell responses

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Background and aims: CD4+ T cells are central regulators of both humoral and cellular immunity. In the context of chronic HBV-infection, HBV-specific CD4 T cells are reduced in numbers and functionally impaired. Since current therapies fail to offer a cure from chronic HBV-infection, novel therapeutic concepts involving immunotherapy are desperately needed. The functional modification of HBV-specific CD4+ T cells may represent a promising approach to improve immunotherapeutic strategies. **Methods:** In order to identify and comprehensively characterize HBV-specific CD4 T cells we expanded PBMCs from chronic HBV patient samples (n = 70) with a pool of overlapping peptides spanning the whole HBV polyprotein followed by flow cytometric analysis for interferon gamma secretion. In parallel, we identified CD4+ T cell responses to Epstein-Barr-virus, influenza A and tetanus toxoid in healthy donors (n = 60). Currently, the addition of agonistic reagents to co-stimulatory receptors and blocking reagents to inhibitory receptors during in vitro culture is analyzed to identify pathways that could improve functionality of HBV-specific CD4+ T cells. **Results:** HBV-specific CD4+ T cell responses were detected in 32% of chronically infected patients, mostly targeting the viral polymerase- and core-antigen region, but not the surface antigen. We are currently evaluating the capacity of inhibitory receptor blockade, addition of cytokines and co-stimulatory molecules to improve the functionality and transcriptional profile of HBV-specific CD4+ T cells and CD4+ T cell responses to control peptides. Our preliminary data suggest a pathogen-specific responsiveness to different culture conditions in vitro. **Discussion:** Understanding the lack of HBV surface antigen-specific CD4+ T-cell responses as well as the identification of pathways that positively influence the antiviral capacities of HBV-specific CD4+ T cells could contribute to the development of a CD4+ T cell based immunotherapy for HBV infection.

5.8 Comparative analysis of CD1d-restricted natural killer T cells in people who inject drugs with chronic or spontaneously resolved hepatitis C

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Background: Natural killer T (NKT) cells represent a subset of immune cells that share characteristics of innate and adaptive immunity. Invariant NKT cells recognize glycolipid antigens such as α galactosylceramide (αGalCer) presented by the non classical MHC molecule CD1d. Decreased NKT cell frequencies have been reported in chronically HCV infected patients, however, contradicting reports exist. We therefore aimed to comparatively study NKT cell frequencies and function in people with chronic and spontaneously resolved HCV infection. **Methods:** CD1d-restricted NKT (CD1d NKT) cells of chronically HCV infected people who inject drugs (PWID) (n = 28) and PWID with resolved HCV infection (n = 33) were analyzed by flow cytometry utilizing a CD1d-tetramer complexed with αGalCer. **Results:** CD1d NKT cell frequencies did not differ between PWID with resolved HCV infection and PWID with chronic HCV infection. Expression of the NK cell receptors NKG2A, NKG2C, NKG2D and KIR2DL3 on CD1d NKT cells, previously described to be differentially regulated on NK cells in HCV infection, was also not significantly different between groups. Interestingly, CD1d NKT cells of chronically infected PWID showed significantly higher expression of the activation marker CD38. Despite this activated phenotype in PWID with chronic HCV infection CD1d NKT cells expressed high levels of CD127 and CD161 and predominantly lacked CD57 irrespective of the infection status. Moreover, no difference in expression of the exhaustion markers PD-1 and BTLA could be observed between groups. Treatment of PBMCs with αGalCer induced robust CD1d NKT cell expansion in both groups associated with upregulation of CD38 and downregulation of CD127. Independent of HCV status, CD1d NKT cells produced similar levels of IFNγ, IL 2, TNFα, IL-4 and CD107a. **Conclusion:** Our data indicate that chronic HCV infection in PWID is associated with increased expression of the activation marker CD38 on CD1d NKT cells, however, this activated phenotype was not associated with differential functionality or altered expression of other NK cell receptors or T cell differentiation markers.

5.9 CRISPR/Cas9 "double"-nickase mediated inactivation of hepatitis B virus replication

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Background: Current antiviral therapies cannot cure hepatitis B virus (HBV) infection, since successful HBV eradication would require the inactivation of the viral genome, which primarily persists in host cells as episomal covalently closed circular DNA (cccDNA) and, to a lesser extent, as chromosomally integrated sequences. However, novel designer-enzymes, such as the CRISPR/Cas9 RNA-guided nuclease system, provide the technology for the development of advanced therapy strategies that directly at-tack the HBV genome. **Methods:** We report here the identification of cross-genotype conserved HBV sequences in the HbS and HbX region of the HBV genome that are specifically and effectively inactivated by a Cas9 double-nickase approach. Pairs of appropriately spaced Cas9 nickase mutants introduced two single-strand breaks on the opposite DNA strands that were subject to NHEJ repair in order to avoid off-target mutations by improving specificity by up to 1,500-fold relative to the wild-type Cas9 enzyme. **Results:** We show that this approach equally inactivated episomal cccDNA as well as chromosomally integrated HBV target sites in reporter cell lines as well as in chronically infected hepatoma cell lines. Analysis of Cas9n activity on ORF S and X target sites by next generation sequencing revealed efficient editing of cccDNA molecules targeted by either gRNA in HBV-infected HepG2.2.15, HepG2-H1.3 and HepG2-NTCP cells. The efficiency of X-specific sgRNAs was particularly high, with approximately 90% of all amplicons reads showing clearly discernible indel signatures. **Conclusion:** Our data support the feasibility of using the CRISPR/Cas9 nickase system for novel therapy strategies aiming to provide a cure for HBV infection.

5.10 Deficiency of Group VIA phospholipase A2 Primes the Cytokine Releases by Kupffer cells and Lymphocytes

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Background and Aims: Impairment of apoptotic cell clearance has been implicated in autoimmunity. We have observed this impairment upon iPLA2β deficiency causing accumulation of apoptotic cells in liver and intestine, and iPLA2β-/- mice were susceptible for injury induced by concanavalinA (ConA) or dextran-sodium-sulfate. Here we aim to determine whether iPLA2β deficiency or combined with CD95/FasL ligation (Jo-2) could alter cytokine release by immune cells. **Methods:** Kupffer cells (KC) or splenocytes and liver lymphocytes were isolated from WT and iPLA2β-/- 3-month-old male mice, and were stimulated by 1 µg/mL LPS or 10 µg/mL ConA, respectively. The stimulation was also studied following treatment with 0.125 µg/g BW Jo-2 for 6 h. Apoptosis and cytokine release were determined by caspase3 activity, cleaved caspase3 staining and ELISA. **Results:** Mutant mice exhibited increased apoptosis in liver and spleen. Mutant KC secreted IL-6, TNF-α, and IL-10 at lower levels than WT KC. However, Jo-2 treatment in vivo primed mutant KC for a marked increase in IL-6 levels, and primed mutant liver lymphocytes for an increased spontaneous release of IFNγ and IL-17. Jo-2 treatment primed splenocytes for increased ConA-stimulated release of IFNγ, and IL-17. **Conclusions:** iPLA2β deficiency caused immunosuppression in KC, but primed splenocytes for Th1/Th17 release. iPLA2β deficiency responded to Jo-2 by sensitizing the release of IL-6 by KC, and Th1/Th17 release by liver lymphocytes. Thus, iPLA2β may regulate cytokine homeostasis in lymphocytes, and its deficiency alone or combined with Jo-2 exaggerated the release of cytokines which may aggravate injury and provoke an onset of autoimmune disease.

5.11 Diminished Antiviral Cytokine Response in Bile Duct Ligated Mice

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Cholestatic patients with high bile acid serum levels are more susceptible to infections and isolated immune cells of these patients behave insensitive against classical pro-inflammatory stimuli. In contrast to these potential proviral properties of bile acids, we recently showed that bile acids reduce mouse cytomegalovirus (MCMV) replication in vitro, thereby exhibiting antiviral potential. To analyse these conflicting properties of bile acids on immunity and virus replication we determined the influence of cholestatic conditions on cytomegalovirus infections in bile duct ligated (BDL) mice. To measure the MCMV induced immune response in liver samples a panel of 18 chemokines and 6 cytokines was quantified by real time PCR. MCMV-induced expression of the pro-inflammatory cytokines and chemokines TNF-α, CCL7, CCL12, CXCL9 und CXCL10 was significantly reduced in BDL compared to sham operated mice. Furthermore, we detected enhanced expression of the anti-inflammatory chemokine IL-10 in MCMV-infected BDL compared to sham operated mice. Consequently, quantification of immune cell infiltration revealed decreased amounts of macrophages in livers of BDL- MCMV-infected mice compared to sham operated MCMV-infected animals. Although bile acids showed anti-inflammatory effects of bile acids in vivo, reduced chemokine/cytokine expression and immune cell infiltration did not lead to increased virus titers in the livers of BDL operated animals. Of note, no titer reduction due to a direct anti-cytomegaloviral effect of bile acids could be observed, indicating its compensation by the pro-viral effect of impaired immune cell infiltration. In summary, bile duct ligation results in an impaired secretion of pro-inflammatory chemokines and cytokines in response to MCMV infection leading to reduced immune cell migration to the liver and thereby most probably counteracting the antiviral activity of bile acids.

5.12 HCV-specific immune responses under direct-acting antiviral therapy of chronic HCV infection

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Background: Direct-acting antiviral drugs (DAA) with or without Ribavirin or PEG-Interferon-alpha efficiently suppress and clear hepatitis C virus (HCV) infection. However, it remains controversial if robust HCV suppression also affects viral immune-escape and restores HCV-directed adaptive immune responses in vivo. **Methods:** In order to address these issues we monitored HCV-specific T-cell response from 20 chronically HCV infected patients throughout treatment with Sofosbuvir, Ribavirin and PEG-Interferon-alpha. HCV-specific CD4 and CD8 T-cell responses were identified by ex vivo HCV epitope pool stimulation and simultaneous detection of 6 functional markers. **Results:** The sustained virological response (SVR) rate was 95 percent, in the study cohort of HCV Genotype 1a/b (n = 7/13) infected patients including cirrhotics (n = 4/16). First results from 10 of 20 treated patients indicate re-activation of HCV-specific CD4 T-cells characterized by TNF-α and IL-2 production at 12 weeks after the end of treatment. However, total cytokine expression by NS3-specific CD8 T-cells was significantly reduced at 12 weeks after the end of treatment compared to therapy initiation. **Discussion:** These data suggest a re-constitution of HCV-specific CD4 T-cells but not of CD8 T-cells following Sofosbuvir, Ribavirin and PEG-Interferon-alpha combination therapy. Further investigations are ongoing to identify HCV-responses during IFN-free DAA regimens, to confirm the HCV-specific T-cell response pattern without therapeutic immune stimulation.

5.13 Hepatitis C virus inhibits IL-1β-induced IκBζ expression in a Calpain- and MK2-dependent manner resulting in LCN2 suppression

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Background: Hepatitis C virus (HCV) infection is a major cause of chronic liver disease worldwide, leading to end-stage cirrhosis and development of hepatocellular carcinoma. To achieve persistence, the virus has evolved several strategies to modulate the inflammatory response, cell proliferation, differentiation and prevent apoptosis. Here, we investigated the influence of HCV-mediated downregulation of the atypical member of the inhibitor of κB protein family IκBζ in response to IL-1β stimulation and its functional consequences focusing on LCN2 deregulation. **Methods:** Huh7 cells either infected with the HCVcc strain JC1 or harbouring the subgenomic HCV1 replicon were used and transcript expression was analysed using rtPCR and immunoblot analysis. Finally, cell proliferation was assessed by the XTT-Assay and the involvement of proteasomal or calpain-dependent degradation was assessed using specific inhibitors. **Results:** Evidence is provided that HCV suppresses IL-1β-induced IκBζ-expression at the protein level via Calpain-dependent degradation. Inhibitor studies further suggest that in hepatocytes induction of IκBζ by IL-1β involves activation of the mitogen activated protein kinase (MAPK) activating kinase (MEK)1/2 and the MAPK activated protein kinase (MK)2. MK2 activation is also reduced by HCV by a yet unknown mechanism, suggesting that HCV thereby interferes with the induction of IκBζ expression. IκBζ is known to act in association with NF-κB as a co-factor for Lipocalin (LCN)2 transcription. IL-1β-induced expression of LCN2 is abrogated in the presence of HCV, an effect that can be mimicked by targeted gene knockdown of IκBζ. Therefore evidence is provided that LCN2 downregualtion is a result of IκBζ suppression. **Discussion:** The data provide a novel mechanism enabling HCV to interfere with the signal-transduction of the inflammatory cytokine IL-1β and to modify the expression of IL-1β-induced genes such as LCN2 via Calpain-dependent degradation of IκBζ and inhibition of the activation of MK2. Since exposure to LCN2 results in a decreased cell proliferation this mechanism may allow HCV to cope with the known proliferation-inhibitory effects of IL-1β on hepatocytes.

5.14 HIV Mono-Infektion ist mit gestörter Anti-HCV-Aktivität von NK-Zellen assoziiert

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Hintergrund: Im Vergleich zu HIV(-) Personen finden sich bei HIV(+) Patienten niedrigere Spontanheilungsraten einer akuten Hepatitis C sowie ein schlechteres Ansprechen auf eine IFN-basierte HCV Therapie. HIV-assoziierte Immundefekte scheinen hierbei eine bedeutsame Rolle zu spielen. In der vorliegenden Arbeit wurde untersucht, wie sich eine HIV-Infektion auf die NK-zellvermittelte Anti-HCV Aktivität auswirkt. **Methoden:** 22 HIV RNA(+) und 29 Patienten unter kombinierter antiretroviraler Therapie (cART) HIV RNA(-) sowie 20 HIV(-) gesunde Kontrollen wurden in die Studie eingeschlossen. Die NK Zell-vermittelte Inhibition der HCV-Replikation wurde anhand des HuH7 HCV-Replicon-Modells analysiert. Die Charakterisierung des NK Zell-Phänotyps sowie der IFN-γ-Produktion von NK-Zellen und der Sekretion von IL-2 durch CD4+ T-Lymphozyten erfolgte mittels Durchflusszytometrie-basierter Analyse. **Ergebnisse:** PBMCs von HIV(+) Patienten zeigten im Vergleich zu gesunden Kontrollen und unabhängig vom Behandlungsstatus eine signifikant verminderte Anti-HCV-Aktivität. Dies korrelierte zum einen mit einem HIV-assoziierten Verlust an NK Zellen und zum anderen mit funktionellen Störungen HIV(+) NK Zellen. So fand sich bei HIV(+) NK-Zellen im Vergleich zu NK Zellen gesunder Personen eine signifikant reduzierte IFN-γ-Produktion, wobei die Frequenz IFN-γ(+) NK-Zellen positiv mit der Hemmung der HCV-Replikation assoziiert war. Zudem fand sich bei den HIV(+) Patienten eine dys-regulierte CD4+ T Zell/NK Zell-Interaktion, die vor allem auf eine gestörte IL-2 Stimulierbarkeit von HIV (+) NK Zellen zurückzuführen war. **Schlussfolgerung:** Eine HIV-Infektion hat einen starken Effekt auf die Anti-HCV-Aktivität von NK Zellen. Dies könnte eine Ursache für den schlechteren klinischen Verlauf der HCV-Infektion bei HIV(+) Patienten darstellen.

5.15 IFN-mediated cytokine induction is associated with sustained virological response in chronic HCV infection

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Hepatitis C virus (HCV) infection is a major cause of chronic liver disease and associated complications such as liver cirrhosis and hepatocellular carcinoma. Interferons (IFNs) are crucial for HCV clearance and a sustained virological response (SVR). There is evidence that an appropriate endogenous IFN response is also required for an efficient final virus clearance by novel directly acting antiviral agents. Understanding the molecular mechanisms of IFN response for the treatment outcome remains therefore important. In this study we found that patients with SVR to IFN-based therapy reveal significantly higher serum levels of TRAIL as compared to patients who failed to control HCV. Upon direct IFNα exposure, PBMCs from SVR patients upregulated TRAIL, IFNγ, CXCL9 and CXCL10 much more strongly than cells from non-SVR patients. As possible mechanism of the stronger IFNα-induced cytokine response, we identified a higher expression and phosphorylation of STAT1 in PBMCs from SVR patients. The increased TRAIL induction additionally involved the NFkappaB and JNK pathway. In conclusion, we could demonstrate that SVR in chronic HCV infection is associated with a strong IFNα-induced cytokine response, which might allow for the early prediction of treatment efficacy.

5.16 IL-12 rather than immune checkpoint inhibitors contribute to the functional restoration of hepatitis D virus-specific T-cells

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Hepatitis D virus (HDV) infection affects 15 – 20 million individuals world-wide and causes severely progressive hepatitis. It is unknown to what extent cellular immune responses contribute to liver disease and why immune responses fail to control viral replication in persistent HDV infection. This study aimed to analyse the mechanisms behind poor immune responses in hepatitis delta. Frequencies of immune cells and specific markers were determined by cell surface stainings of PBMCs, derived from chronic HDV patients and from healthy controls (HDV n = 49; Healthy n = 18). Cell proliferative capacity was characterized by 3 H thymidine assays (n = 45) while functionality was determined by intracellular cytokine stainings. Samples were stimulated with 15mer peptide sequences derived from the large hepatitis delta antigen overlapping by 11 amino acids. To restore T cell responses, either blocking antibodies (CTLA-4, PDL-1) or pro-inflammatory cytokines (IL-12) were used. We show that T-cell subsets were not altered in HDV patients compared to healthy controls. However, the senescence marker CD 57 was significantly upregulated on CD 8+ T-cells in hepatitis delta patients. Subsequently, weak and very infrequent HDV-specific T-cell proliferative and cytokine responses were observed by in vitro stimulation with overlapping peptides covering the entire HDV antigen. Blocking the co-inhibitory molecule PD 1 and to a lesser extent also CTLA-4 slightly restored HDV-specific T-cell immunity in some cases. In contrast, a more robust and consistent increase in both HDV-specific CD 4+ and CD 8+ T-cell responses was evident when the third signal cytokine IL-12 was added in vitro. **Conclusions:** This so far largest investigation of virus-specific T-cell immunity in patients with hepatitis delta revealed premature aging of immune cells associated with largely impaired T-cell functionality in chronic HDV infections. However, weak proliferation and cytokine production could be restored by blocking inhibitory pathways and in particular supplementation of third-signal cytokines. Overall, these data give new insights into the immunopathogenesis of hepatitis delta and supports the development of immunomodulatory treatment strategies.

5.17 Lack of HBeAg does not induce stronger enhancement of innate immunity genes in humanized mice

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The HBeAg of HBV was reported to counteract immune responses whereas the precore mutation G1896A, which abolishes HBeAg production, has been associated with increased HBV replication. Nevertheless, the role of HBeAg in the HBV life cycle in vivo and its capacity to modulate the expression of antiviral innate signaling pathways remains unclear. Aim of the study was to investigate HBV infection kinetics, replicative activity and expression of innate immunity genes in humanized mice infected with virions lacking HBeAg expression in comparison to wild type (wt) virus. **Methods:** Mice with comparable levels of human liver chimerism were inoculated with cell culture-derived wt HBV (genotype D) or its derived variant harboring the G1896A precore mutation. Viremia development was monitored at 3, 6, 9 and 12 weeks post infection. Levels of HBeAg were confirmed by CMIA (Abbott Architect). Intrahepatic virological parameters and expression levels of human and murine genes were analyzed using qPCR. To assess whether HBeAg is able to interfere with the TLR2 mediated innate immune response, mice were stimulated once with the TLR2 agonist Pam3Cys and analyzed either after 2 h, 4 h or 8 h. **Results:** HBV viremia development did not differ significantly among the wild type (n = 6) and the HBeAg-negative group (n = 5), showing median titers of 8E+4 and 3E+4 copies/ml at week 3, respectively and reaching median values of 1xE+8 and 3E+8 HBV DNA copies/ml at week 12 post infection. Intrahepatic amounts of pgRNA, rcDNA and cccDNA estimated per human hepatocyte appeared slightly higher in mice harboring the precore mutant variant (n = 6) in comparison to the wt group (n = 8). Nevertheless, analysis of selected human interferon stimulated genes (i. e. hMX1, hISG15, hISG20, hOAS 1, hUSP18), genes involved in TLR signaling (i. e. hTLR2, hTLR3, hMYD 88) and cytokines (hIL 6, hCXCL 10, hTGFb, hIFNa, hTNFa), as well as murine genes (mTlr's, mIl6, mTgfb, mIfnb, mCxcl10, mMx1), revealed that expression levels did not differ among mice infected with wt or G1896A viruses, although some human genes (i. e. hCXCL 10, hTAP1) appeared) moderately increased in both groups of infected mice in comparison to uninfected controls (n = 7). Pam3Cys administration induced strong enhancement of murine (i. e. mTlr2, mTnfa) and to a lesser extent of human genes, regardless of the HBeAg status, while viremia and intrahepatic pgRNA levels remained unaffected. **Conclusion:** Although infection kinetic studies did not reveal different spreading capabilities between HBeAg-positive and -negative viruses, intrahepatic viral loads were slightly higher in mice stably infected with the precore mutant. Surprisingly, the lack of HBeAg did not induce stronger innate responses or facilitate TLR signaling induction in infected mice, thus suggesting that at least in the absence of adaptive immune responses, the precore protein does not contribute to dampen infection recognition in HBV-infected primary human hepatocytes.

5.18 Mechanistic dynamics of Hepatitis C virus replication in single liver cells

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Infection with hepatitis C virus (HCV) causes chronic liver diseases. Strong biological evidence suggests intracellular spatial dependence is a crucial factor in the process the virus uses to replicate its RNA genome. For HCV, replication is believed to occur in specialized compartments within virus-infected cells, termed replication complexes. Replication complexes are derived from altered regions of an interconnected intracellular membrane network called the Endoplasmic Reticulum (ER). The HCV-encoded NS 5A protein is an essential component of HCV replication and probably contributes many functions that the virus is dependent upon to replicate its RNA and assemble its progeny. Research has revealed a substantial spatial facet of NS 5A function and particular biophysical characteristics of the protein arise from its anchoring to the 3D embedded curved 2D ER manifold. To facilitate discovery of novel anti-HCV treatments, it is necessary to understand the dynamics of virus replication within human liver cells. Computational virology is a relatively new field and aims to describe the physics underpinning virus replication using mathematical formulae. An approach such as this may reveal areas of the virus life cycle amenable to novel antiviral intervention that conventional biology may miss e. g. spatial dependence of virus-encoded factors within specific intracellular regions. Exploring the biophysics of viral replication mechanism through cross-discipline work, i. e., application of physics-based solutions to understand biology-based data is a highly interesting aim. We used data derived from 3D confocal microscopy of HCV-infected human hepatoma cells labeled for the ER membrane in order to reconstruct 3D geometries of single hepatocytes using NeuRA2. On top of these geometries, we developed a model using (surface) partial differential equations (sPDE) of viral RNA replication dynamics with particular emphasis upon RNA movement, viral protein production, cleavage and movement, and viral RNA replication within the membranous web. In particular, we present the estimation of the biophysical meaningful NS 5A diffusion constant based on the comparison of experimental FRAP time series data and simulation data. Advanced simulations within biophysical applications ask for advanced algorithms and implementations which are running efficiently on massively parallel high performance computers. The arising sPDEs on the ER surface are solved using the simulation platform UG4 within a Finite Volume framework combined with multigrid techniques. The application of modern scientific computing technology to our advanced biophysical concepts for solving challenging real-life problems paves new ways for computational virology.

5.19 Assoziation des IL28B Polymorphismus mit dem Ausmaß der Monozyten-induzierten NK Zell-Aktivierung bei der Hepatitis C

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Hintergrund: Immunogenetische Studien weisen auf einen Zusammenhang zwischen NK Zellen und Lambda-Interferonen (λ-IFNs) wie IL28B hin. Allerdings exprimieren NK Zellen den λ-IFN Rezeptor nicht und reagieren passend hierzu auch nicht auf eine Stimulation mit λ-IFNs. Daher vermuteten wir eine eher indirekte Assoziation von IL28B Genotyp und NK Zell-Aktivität. **Methoden:** Bei 31 HCV(+) Patienten und 42 gesunden Kontrollen wurden der IL28B Genotyp (rs 12979860) mittels rtPCR bestimmt. PBMC isolierte Monozyten bzw. NK Zellen wurden mit IL28B und/oder dem TLR7/8 Agonisten R848 stimuliert. Die Produktion von IFN-γ, IL-12 bzw. IL-18 wurde mittels FACS oder ELISA bestimmt. In Blockierungsexperimenten wurden Anti-Il12 und/oder Anti-IL18 verwendet. **Ergebnisse:** Nach Stimulation von PBMC mit R848 zeigten Träger eines T/T IL28B-Genotyps die niedrigsten Frequenzen IFN-γ(+) NK Zellen. Allerdings fand sich eine solche Assoziation nicht, wenn isolierte NK Zellen analysiert wurde, was auf einen indirekten Zusammenhang hinwies. In Übereinstimmung hiermit fanden wir, dass Monozyten von Patienten mit einem T/T Genotyp die IFN-γ Produktion von NK Zellen signifikant schwächer stimulierten, als dies bei Patienten mit einem non-T/T Genotyp der Fall war. Passend hierzu fanden wir, dass Monozyten von T/T Patienten signifikant weniger IL-12 sezernierten als Monozyten von Patienten mit einem non-T/T Genotyp. Bezüglich der IL-18 Produktion wurden keine Unterschiede zwischen den Genotypen festgestellt. In Blockierungsexperimenten konnte eine wichtige Rolle dieser Zytokine für die Monozyten-induzierte NK Zell Aktivierung bestätigt werden. Interessanterweise fand sich diese Assoziation zwischen IL28B Genotyp und Monozyten-vermittelter Stimulation von NK Zellen nur bei HCV-Patienten jedoch nicht bei gesunden Kontrollen. **Schlussfolgerung:** Unsere Daten bieten eine mögliche Erklärung für den Zusammenhang von NK Zell Funktion und IL28B Genotyp.

5.20 Quantification of serum HBV RNA allows differentiation of disease stages of hepatitis B virus (HBV) infections

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Introduction: Inactive carriers of HBV infections are distinguished from individuals with active HBV infections by serum levels of HBV DNA. HBV RNA is another surrogate marker of HBV replication which can be quantified from serum. The aim of this study was to explore whether HBV RNA levels permits a better differentiation between the various stages of HBV infection. **Methods:** Serum samples from 12 patients with acute and 96 with chronic HBV infection (34 inactive HBsAg carrier with HBV DNA < 4 log10 copies/ml and ALT within normal ranges, 34 HBeAg negative and 28 HBeAg positive patients with chronic active hepatitis B (mean age 45.4 ± 15.6 (17 – 77) years, m/w = 73/35, mean ALT 8.7 ± 19.2 (0.22 – 100) µkat/ml, mean HBsAg 4.3 ± 0.9 (0.7 – 6.0) log10 ng/ml) were retrospectively analyzed. HBV DNA was measured by real-time PCR (limit of detection 2.6 log10 copies/ml). HBV-RNA levels were quantified based on a sensitive One-Step-RACE-PCR-Assay which was developed and validated (limit of detection 2.9 log10 copies/ml). **Results:** HBV RNA could be detected in 9 of 12 patients with acute HBV infection with a mean of 3.4 ± 1.6 (2.0 – 5.9) log10 copies/ml. HBV RNA was detectable in 29 out of 34 HBeAg negative patients and in all 28 patients with HBeAg positive chronic active HBV infection and showed a high correlation with HBV DNA levels (r = 0.93). Mean HBV DNA levels were significantly lower in HBeAg negative as compared to HBeAg positive patients (6.8 ± 1.3 (4.2 – 8.9) vs. 8.2 ± 1.4 (4.5 – 10.5) log10 copies/ml, $p < 0.001$), and so were mean HBV RNA levels (4.4 ± 1.5 (2.0 – 7.0) vs. 6.8 ± 1.2 (4.3 – 9.1) log10 copies/ml, $p < 0.001$). In contrast, HBV RNA was detectable in only in 2 out of 34 inactive HBsAg carriers (2.0 ± 0.2 (2.0 – 2.7) log10 copies/ml), while in 28 of those patients HBV DNA was still detectable with a mean of 3.1 ± 0.7 (2.0 – 4.6) log10 copies/ml. **Conclusion:** Serum levels of HBV RNA show a high correlation with HBV DNA levels in patients with active chronic HBV infection. However, in patients with inactive HBV infections, HBV RNA is less frequently detected than HBV DNA. It needs to be studied whether HBV RNA serum levels can identify patient inactive carrier status that have disease progression.

5.21 Redundant tolerance mechanisms prevent autoimmune liver inflammation in mice

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Background: The role of Foxp3+ regulatory T cells (Tregs) in autoimmune liver inflammation is still being discussed. Whereas some reports suggested that autoimmune liver inflammation is linked to Treg dysfunction, others could not confirm this observation. **Aims:** Here, we investigated the consequences of Treg or IL-10 impairment for the development of autoimmune hepatitis. We used a mouse model that is characterised both by ectopic expression of the prototypical autoantigen myelin basic protein (MBP) in the liver and by the presence of autoreactive MBP-specific T cells due to a transgenic T cell receptor. **Methods:** The functionality of T effector cells in (CRP-MBP x tg4) mice was confirmed in vivo and in vitro. Treg impairment in these mice was induced either by application of a Treg depleting anti-CD25 antibody, or by crossing with hCD2-ΔkTβRII mice. In these mice, T cells feature a dominant-negative TGFβ receptor that prevents peripheral Treg induction. Moreover, the role of IL-10 for the maintenance of hepatic tolerance was assessed in (CRP-MBP x tg4 x dnIL10R) mice, in which T cells are insensitive to IL-10. Alternatively, anti-IL10 receptor anti-body was administered to (CRP-MBP x tg4) mice. **Results:** (CRP-MBP x tg4) mice were completely resistant to the induction of autoimmune inflammation in liver and CNS. MBP-specific effector cells of (CRP-MBP x tg4) mice were functional, as indicated by their capability to secrete IL-17 (371.0 pg/ml vs. 191.0 pg/ml of wildtype tg4 cells) and IFNγ (339.8 pg/ml vs. 429.2 pg/ml of wildtype tg4 cells). Moreover, (CRP-MBP x tg4) mice manifested elevated Treg numbers in spleen (10.5% CD4+Foxp3+ vs. 8.12% of wildtype tg4 cells) and liver (12.67% CD4+Foxp3+ vs. 6.33% of wildtype tg4 cells). However, neither Treg depletion with anti-CD25 antibody in (CRP-MBP x tg4) mice nor Treg impairment in (CRP-MBP x tg4 x hCD2-ΔkTβRII) precipitated autoimmune inflammation of liver or CNS. Upon adoptive transfer into CRP-MBP mice, MBP-specific CD4 T cells differentiated into CD49b+Lag3 + Tr1-like cells. However; impairment of IL-10 signalling did not lead to autoimmune inflammation in liver or CNS. Even simultaneous abrogation of both Treg and IL-10 signalling in (CRP-MBP x tg4 x hCD2-ΔkTβRII) mice treated with anti-IL10R antibody or (CRP-MBP x tg4 x dnIL10R) mice treated with anti-CD25 antibody did not result in autoimmune inflammation in liver or CNS. **Conclusion:** Our findings show that neither Treg impairment nor the abrogation of IL-10 signalling alone or in combination do result in the development of autoimmune hepatitis. Thus, redundant, but yet undefined tolerance mechanisms safeguard the maintenance of hepatic tolerance.

5.22 The breadth of CD8+ T cell responses in chronic and resolved HBV infection

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Introduction: Approx. 350 million people are chronically infected with hepatitis B virus (HBV) worldwide. HBV-specific CD8+ T cells are crucial for viral clearance. T cell based immunotherapies are thus a promising strategy for clearance of chronic HBV infection. However, little is known about HBV-specific CD8+ T cell epitopes. Therefore, we aim to identify novel HBV-specific CD8+ T cell epitopes and to differentiate the CD8+ T cell repertoire in chronically HBV infected patients compared to patients who spontaneously eliminated the virus. **Methods:** PBMC from 70 patients chronically infected with HBV genotype D and 16 patients with resolved HBV infection were screened in an ELISpot-Assay using overlapping peptides (OLPs, 18mers) covering the full HBV genotype D proteome. Positive ELISpot responses were confirmed by intracellular interferon-γ (IFN-γ) staining. Afterwards, epitopes were finemapped and HLA-restriction was determined using EBV-immortalized B cells. Additionally, viral sequences were determined in chronically infected patients and HLA associated sequence polymorphisms ("footprints") were identified as indicators for escape mutations. **Results:** Both the immunological

and the virological analysis revealed new HBV-specific CD8+ T cell epitopes. Interestingly, chronically infected patients targeted epitopes in polymerase, core- and X-protein but not in HBsAg, whereas patients with resolved HBV infection additionally targeted epitopes in HBsAg. 50% of the patients showed at least one CD8+ T cell response to an OLP. In total, 70 epitopes could be detected. Furthermore, most of the footprints were found in polymerase and core, but only few and weak footprints were found in HBsAg. This goes along with the lack of IFN-γ responses in the HBsAg, indicating that in patients with chronic HBV infection, CD8+ T cell responses do not target HBsAg and thus do not drive viral escape in this viral region. **Conclusion:** By performing both a comprehensive analysis of HBV-specific CD8+ T cell responses as well as analysis of HLA class I footprints, we were able to identify novel HBV-specific CD8+ T cell epitopes and to demonstrate viral escape in some of these epitopes. In addition, the lack of responses to HBsAg in patients with chronic infection implies a dominant role of the HBsAg in immune regulation and persistence of the virus.

5.23 The diverse functions of transcription factors in T cell immunity during LCMV infection

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We aimed to decipher the role of IRF4 and its cooperating binding partner BATF and STAT3 in virus-specific CD8+ and CD4+ T cell immune function. Our results demonstrate that IRF4 and BATF but not STAT3 are necessary for sustained CD8+ T cell effector function. IRF4 and BATF deficiency resulted in limited CD8+ T cell responses after LCMV infection. Consequently, Irf4-/- and BATF-/- mice established chronic infections, but were protected from fatal immunopathology. These data identify the transcription factors IRF4 and BATF as major regulators of anti-viral cytotoxic T cell immunity. Mice with conditional disruption of STAT3 in T cells have an impaired generation of T-follicular helper (Tfh) cell. STAT3 T cell deficient mice did not accumulate bone marrow virus specific IgG-secreting cells or mount neutralizing responses to LCMV. Our results demonstrate the importance of STAT3 in T cells for the generation of functional long-term humoral immunity to viral infections.

5.24 The Hepatitis C Virus (HCV) results in an increased attraction of human neutrophils to its host cell by a basal and EGF-induced CXCR2 ligand expression

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Introduction: To modulate the inflammatory response of the host and to utilize host infrastructure without largely impeding viability of the infected cell HCV requires strategies to establish persistent infections and to escape antiviral immunity. Chemokines have the major function as chemoattractants to lead the migration of immune cells. HCV has evolved mechanisms to gain access to the control of these factors. The present study investigates the impact of HCV on the expression of the CXCR2 ligands CXCL1 to 3 and 8 in its host cell, the ability of EGF to induce these mediators and the biological function of the same. **Methods:** Huh-7 cells either infected with the HCV cc strain JC1 or harbouring the HCV subgenomic replicon were used and the impact of HCV on cellular signalling and gene expression was analysed employing targeted gene knockdown by siRNA, inhibitor-studies, rtPCR and Immunoblot-analysis. Human neutrophils were isolated from whole blood from healthy donors by a density gradient separation from other blood cells and were used for migration assays. **Results:** In the presence of the HCV replicon or of the HCV strain JC1 the expression of the CXCR2 ligands CXCL1, 2, 3 and 8 in response to EGF was significantly increased. Inhibitor studies and data from gene knockdown experiments using specific siRNA suggests that sensitization of EGF receptor (EGFR) tyrosine kinase activity by HCV-mediated reduction of the negative regulator of EGFR the T cell protein tyrosine phosphatase (TC-PTP) is responsible for increased EGF-induced chemokine expression in HCV infected cells and that activation of MEK1 and NF-κB is involved in CXCL8 expression. Most interestingly gene knockdown by siRNA suggests that HCV itself induces and enhances production of these chemokines via induction of EGF expression by an autocrine circuit in its host cell. Migration assays and CXCR2-inhibitor studies suggest an enhanced neutrophil attraction by HCV to its host cell, which could be significantly increased in response to EGF-induced chemokine expression of the virus. **Conclusion:** The study provides evidence, that HCV enhances the expression of the chemokines CXCL1 to 3 and 8 by an EGF-mediated autocrine circuit, which can be further enhanced by addition of external EGF. This may enable HCV to influence the recruitment of inflammatory cells or immune cells, such as neutrophils, thereby modulating the local inflammatory and immune microenvironment or to increase to viral replication.

5.25 The HLA-DPA1 rs3077 TT polymorphism is associated with spontaneous resolution of hepatitis B virus (HBV) infections in Caucasians

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Background: Human leukocyte antigens (HLA) play an important role in the regulation of immune response against infectious organisms, including HBV. Single nucleotide polymorphisms (SNPs) within the HLA-DP locus were recently found to be associated with HBV infection and viral clearance in Asian populations, but little is known about implications of these variants for the course of HBV infection in Caucasians. We aimed to investigate the impact of HLA-DPA1 rs3077 and HLA-DPB1 rs9277534 polymorphisms on the likelihood of spontaneous HBsAg clearance in a mainly European population. **Methods:** 435 chronic hepatitis B (CHB) patients (CHB group) and 111 subjects with spontaneous HBs antigen clearance (SC group) defined by undetectable HBsAg and detectability of anti-HBs and anti-HBc IgG were enrolled (465 European Caucasians, 61 Asians and 20 Africans). The Caucasian subjects descended from northern/eastern Europe (n = 352), the Mediterranean region (n = 106) or the Middle East (n = 7). Genomic DNA was extracted from peripheral blood samples. HLA-DPA1 rs3077 and HLA-DPB1 rs9277534 single nucleotide polymorphisms were genotyped by polymerase chain reaction and melting curve analysis. Comparison between the two groups was made using Chi-square test and logistic regression analysis. **Results:** In the CHB group, 28.5% were inactive carriers, 67.7% were HBe-antigen negative, 17.2% had cirrhosis, 6.5% had hepatocellular carcinoma and 4.4% were liver transplanted. 85.6% had already received treatment for CHB. The mean age and sex distribution differed significantly between the CHB and the SC groups (45.38 ± 15.4 vs. 61.35 ± 14.5; $p < 0.0001$ and 65.9% vs. 55.5% for men; $p = 0.036$, respectively). Therefore, all analyses were age and sex adjusted. There was no significant association found for HLA-DPB1 rs9277534 polymorphisms between the two groups. However, the HLA-DPA1 rs3077 non-TT (CT or CC) genotype was significantly more frequent in CHB patients as compared to the patients in the SC group (43.6% vs. 25.5%; $p = 0.008$, OR = 2.029, 95% CI = 1.202 – 3.425). When the European patients were separately analyzed (359 with CHB and 106 with SC), this association remained statistically significant ($p = 0.022$, OR = 1.938, 95% CI = 1.102 – 3.409). In the CHB group neither HLA-DPA1 rs3077 nor HLA-DPB1 rs9277534 polymorphisms were associated with inactive carrier status, HBe-antigen status or liver cirrhosis. **Conclusions:** This is the first study demonstrating an association of the HLA-DPA1 rs3077 TT genotype with clearance of HBV infections in a Caucasian population. The use of this marker should be assessed for individualized risk assessment.

Namenverzeichnis