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Falk Workshop
Pathophysiology and Treatment of Cholangiocarcinoma

January 23 – 24, 2014
Universitätsklinikum Tübingen
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Abstracts
Poster Abstracts
Abstracts of Invited Lectures
Poster Abstracts

Falk Workshop

PATHOPHYSIOLOGY AND TREATMENT OF CHOLANGIOCARCINOMA

Tübingen (Germany)
January 23 – 24, 2014

Scientific Organization:
A. Königsrainer, Tübingen (Germany)
N.P. Malek, Tübingen (Germany)
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Session I

Molecular basis of biliary cancer formation
Development and plasticity of biliary cells

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Molecular mechanisms driving cell differentiation in embryonic liver may be reactivated in adult disease. Hence, studying the mechanisms of hepatic differentiation in the embryo is relevant to understand adult pathogenesis.

During liver development hepatic progenitor cells, called hepatoblasts, differentiate to the hepatocyte or cholangiocyte lineage. Signaling pathways promoting differentiation of cholangiocytes include TGF-beta, Notch, Wnt, Hippo, FGF and BMP signaling. Our laboratory contributed to characterize the role of TGF-beta and Notch signaling. Others showed that activation of Notch signaling in adult liver can stimulate transdifferentiation of hepatocytes to cholangiocytes, thereby mimicking embryonic development of cholangiocytes. Importantly, such transdifferentiation may constitute an early stage in the pathogenesis of intrahepatic cholangiocarcinoma. We will present our recent data on the function of Wnt signaling in cholangiocyte differentiation.

Moreover, our lineage tracings of embryonic cholangiocytes revealed an unexpected plasticity: while embryonic cholangiocytes generate bile ducts, as expected, a fraction of these cells revert to a hepatocyte phenotype around birth. Embryonic cholangiocytes also give rise to cells lining the canals of Hering, where adult liver progenitors are considered to be located. Our further lineage tracing experiments in adult liver demonstrated that cells emanating from the bile ducts or from the canals of Hering can be activated upon injury and differentiate to mature hepatocytes.

We conclude that both in embryonic and adult liver, the cholangiocyte lineage is characterized by bipotentiality.
The role of stem cells in cholangiocarcinoma

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Stem cells are particularly prone to be involved in the carcinogenic process. Two types of stem cell niches have been so far identified in the liver. The first type, located in the canals of Hering and bile ductules, has been considered in the origin of mixed-cholangiocarcinoma (mixed-CCA). The second type, located in peribiliary glands, is composed of multipotent stem cells of endodermal origin (hBTSCs). Recent evidence suggests that hHpSC are involved in the pathogenesis of pure mucin-CCA emerging from the extrahepatic biliary tree or from the large intrahepatic ducts. Therefore, mucin-CCA could be considered a cancer of peribiliary glands, sharing similarities with colorectal cancer arising from colon crypts and with pancreatic cancer originating from the glands of pancreatic ducts. Recently, we demonstrated that different subpopulations of cancer stem cells (CSCs) are present in CCA. However, while mucin-intrahepatic and extrahepatic-CCA are similar, a different profile of CSCs characterizes mixed and mucin-CCA thus confirming their patho-biological diversity. Specifically, CD13+ CSCs predominated in mixed-CCA while CD90+ in mucin-CCA. In subcutaneous tumor xenografts, cancers with a large predominance of stromal markers were formed by mesenchymal (CD90+) or epithelial (CD133+) CCA CSCs. In intrahepatic xenografts, in contrast, cancers with predominance of epithelial features were formed. Remarkably, CD133+ CSCs, injected in the cirrhotic liver, reproduced the pure mucin-CCA while CD90+ CSCs formed undifferentiated CCA. In conclusion, CSCs are heterogeneous in human CCA and generate different type of cancers depending from the type of CSC and the microenvironment. Remarkably, we identified a single CSC (CD133+) reproducing the mucin-CCA when injected in the cirrhotic liver.
Cholestasis – A necessary pre-requisite for cholangiocellular carcinoma formation?

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Cholangiocarcinoma (CCA) is the second most frequent malignancy of the hepatobiliary tract. CCA is classified as intrahepatic (iCCA), perihilar (pCCA) and distal (dCCA) depending on the localization of the tumor. The risk profile for the development of CCA is different in areas of high incidence of CCA such as Southeast Asia and those with low incidence such as Europe or the US.

In the Far East, biliary tract infections with Clonorchis sinensis and Opisthorchis viverrini are the most frequent disorders predisposing to development of CCA. It is under discussion whether anthelminthic treatment with praziquantel may even enhance the risk for CCA development in infected patients. Oriental hepaticolithiasis represents the second relevant predisposition to CCA development in the Far East.

In contrast to Southeast Asia, Western populations in the US and Europe are barely exposed to helminthic infections of the biliary tract. Here, immune-mediated disorders and malformations play a more important role. Primary sclerosing cholangitis (PSC) represents a leading predisposition to development of CCA with an annual risk of 0.5–1.5% and lifelong prevalence of up to 10%. More recently, a large population-based case-control study in the US disclosed – next to established risk factors like choledochal cysts, cholangitis, and inflammatory bowel disease – a number of other risk factors for iCCA as well as pCCA/dCCA including biliary cirrhosis, cholelithiasis, alcoholic liver disease, cirrhosis in general, diabetes, thyreotoxicosis or chronic pancreatitis. In addition, obesity, HBV and HCV infection and smoking appear to be predisposing to iCCA. A number of other predisposing disorders of the biliary tree such as cystic fibrosis, congenital hepatic fibrosis, Caroli disease, polycystic liver disease or biliary hamartomas (von Meyenburg complexes) have been discussed as risk factors mainly for iCCA.

Looking at these diverse risk profiles, cholestasis does not appear to be mandatory for CCA formation, but may contribute to CCA formation. In particular, hydrophobic human bile acids may need attention in the pathogenesis of CCA. Hydrophobic bile acids are potent activators of the epidermal growth factor receptor (EGFR) and mitogen-activated protein kinases and may induce cyclooxygenase 2 over-expression. These stimuli might play a role in the induction of proliferative responses in cholangiocytes.

Thus, cholestasis does not present a ‘conditio sine qua non’ for CCA formation, but may provide a pro-proliferative milieu in which development of CCA is facilitated.

References:


Session II

Predisposing conditions
The genetics of primary sclerosing cholangitis

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Different avenues are currently being pursued to determine the pathophysiological basis of primary sclerosing cholangitis (PSC). Like in most inflammatory phenotypes, the application of the genome-wide association study (GWAS) design to large collections of patients and controls has been successful, and has led to the identification of 16 genetic loci associating with PSC development. A general theme of the outcome of these studies is that most of the PSC loci also associate with other inflammatory and autoimmune conditions. One group of loci represent key immune pathways with vast prior knowledge on biology (e.g. IL2, IL2RA). For another group of loci, less prior knowledge on implicated biology exist (e.g. TCF4, GPR35). Finally, for some of the loci (e.g. MST1, HLA) determining the most important gene has proven difficult, meaning biological interpretation is difficult. The basis for the relationship between genetic associations and pathobiology in PSC has not been firmly established for any of the loci.

Intrinsic to the GWAS study design, associated variants are common (frequency in the healthy population of 5% or more), meaning biological alterations are likely subtle. Since only in the presence of complex gene-gene and gene-environment interactions these biological alterations surface as clinical disease, this also means that many of the concepts deriving from monogenous traits (e.g. ABCB4 and cystic fibrosis-associated cholangiopathy) are most likely not valid in exploring the gene-disease relationship. For some of the loci (e.g. HLA and FUT2), determining the nature of interacting environmental factors may prove equally important as the biological function of the gene products per se. As an example, for the FUT2 locus, this means to determine the impact of an altered gut microbiota on immune function and bile acid metabolism.

In the current presentation, an update on the genetics of PSC will be given, including an overview of ongoing studies and future prospects. In addition, general reflections upon gene-biology relationships for the PSC susceptibility loci will be made, with a particular emphasis on the importance of gene-environment interactions.
Biliary stones and cholangiocarcinoma formation

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Gallbladder stones are predominantly composed of cholesterol (and a bilirubin nucleus) and are caused by biliary hypersecretion of cholesterol. They may lead to chronic cholecystitis, which is associated with an increased risk of gallbladder cancer, in particular in the setting of selective mucosal calcifications [1, 2]. Hence, all hereditary factors such as the hepatobiliary cholesterol transporter variant $ABCG8$ p.D19H and environmental conditions predisposing to gallbladder stones also predispose to gallbladder cancer [3, 4]. Accordingly, most of the patients with gallbladder cancer are older than 60 years, and 70–90% of the patients have concurrent gallstones. The absolute incidence of gallbladder cancer determines cost effectiveness of prophylactic cholecystectomy, which is indicated in high-incidence countries such as Chile [5, 6].

Bile duct stones can lead to chronic cholangitis, which predisposes to (intra- and extrahepatic) cholangiocarcinoma. Intrahepatic brown pigment stones composed of calcium bilirubinate are common in the Far East. Hepatolithiasis often coincides with parasite infestations (Clonorchis sinensis, Opisthorchis viverrini) and can present clinically as recurrent pyogenic cholangitis (RPC). Intra- and extrahepatic bile duct stones are often associated with other conditions leading to choledochal cysts, biliary obstruction and stasis such as Caroli syndrome or low-phospholipid associated cholelithiasis (LPAC) due to mutations of the hepatobiliary phospholipid transporter $ABCB4$ [7]. Accordingly, these conditions might also confer bile duct stone and cholangiocarcinoma risk, as highlighted by cholangiocarcinoma formation in children with severe transporter variants [8–10]. Of note, pancreaticobiliary maljunction allows regurgitation between bile and pancreatic ducts and predisposes to biliary cancers, and intraductal papillary mucinous neoplasms of the pancreas [11].

References:


Inflammatory biliary disease – From mouse models to new treatment options

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The etiology and pathogenesis of chronic inflammatory bile duct diseases/cholangiopathies such as primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) are still poorly understood. Mouse models of chronic inflammatory bile duct diseases are critical for improving our understanding of the pathogenesis and advancing treatment of these disorders. While ursodeoxycholic acid (UDCA) is an established treatment option for PBC (with the drawback of incomplete responders in about one third of cases), no established medical treatment for PSC is available, reflecting our shortcomings in understanding the pathomechanisms of this disease. Several spontaneous mouse models for PBC (not requiring previous manipulations for breakdown of immunotolerance to pyruvate dehydrogenase (PDC)-E2 protein) have been reported including NOD.c3c4 and NOD.c3c4-derived mice, IL-2Rα−/− mice, dominant negative TGF-β receptor II mice and Ae2a,b−/− mice. While these mouse models reproduce some of the immunological abnormalities and impairment of the biliary bicarbonate umbrella in PBC, they have been of only limited use for development of new treatment options.

PSC is frequently associated with inflammatory bowel disease (IBD; 70% to 80% of cases) and leads to end-stage liver disease requiring liver transplantation. PSC is also a premalignant condition associated with an increased risk for cholangiocarcinoma (CCA) and colorectal cancer (CRC). Since effective medical therapy is still lacking, PSC is a potentially fatal disease with poor prognosis (median transplant-free survival of 12 to 18 years; 10-year survival: approximately 65%). To date, no animal model has been developed that exhibits all of the attributes of PSC. Mice with targeted disruption of the Mdr2 (Abcb4) gene encoding a canalicular phospholipid flippase (Mdr2−/− mice) spontaneously develop sclerosing cholangitis with macroscopic and microscopic features of human PSC. Bile duct injury in these mice is linked to defective biliary phospholipid secretion resulting in an increased concentration of free non-micellar bile acids which subsequently cause bile duct epithelial cell (cholangiocyte) injury, pericholangitis, periductal fibrosis with ductular proliferation and finally sclerosing cholangitis. In analogy to the Mdr2−/− mouse model of sclerosing cholangitis, MDR3/ABCB4 (the human orthologue of rodent Mdr2/Abcb4) defects could play a role in the pathogenesis of various cholangiopathies in humans. MDR3 variants could play a role as modifier gene in the pathogenesis of various cholangiopathies such as PSC, PBC and adulthood idiopathic ductopenia / biliary fibrosis. One of the major drawbacks of the Mdr2−/− mouse model is the lack of IBD and CCA.

Nevertheless, the Mdr2−/− mouse model of cholestasis and cholangitis has proven very useful to test novel treatment strategies for cholangiopathies. norUDCA (but not “conventional” UDCA) reversed sclerosing cholangitis in the Mdr2−/− cholangiopathy model within 4 weeks of treatment. norUDCA is a side chain-
shortened C23 homologue of UDCA is more resistant to conjugation with taurine or glycine than UDCA, but instead is secreted into bile mostly in unchanged form. The secreted norUDCA undergoes cholehepatic shunting which leads to a bicarbonate-rich hypercholeresis and may also result in improved targeting to the liver and dicideae bile ducts. Its possible therapeutic mechanisms in treatment of cholestasis and choangitis include (i) amelioration of bile hydrophobicity by biliary enrichment with hydrophilic norUDCA and its metabolites, (ii) flushing of injured bile ducts by stimulation of bile flow and bicarbonate-rich choleresis, which dilutes toxic biliary content and reinforces the bicarbonate umbrella protecting against potentially toxic bile acids, (iii) induction of alternative bile acid detoxification (phase I and II enzymes) and elimination routes for bile acids, and (iv) direct anti-inflammatory and anti-fibrotic properties. Notably, tauro-norUDCA which lacks cholehepatic hepatic shunting with stimulation of bicarbonate secretion also looses the therapeutics effects. A recent comprehensive gene expression and metabolomic profiling revealed profound alterations in fatty acid and triglyceride metabolism, including a restoration of elevated short-chain and medium-chain fatty acids and reduced long-chain fatty acids resulted in a less lipotoxic lipid profile in the Mdr2−/− cholangiopathy model by norUDCA. norUDCA also targets the inflammatory cross talk between cells involved in inflammation and fibrogenesis in sclerosing cholangitis. As such, norUDCA represents a multi-targeted therapeutic approach, targeting hepatocytes, cholangiocytes and Kupffer cells. Such a multi-targeted therapeutic approach may be essential for the treatment of a complex multifactorial disease such as PSC, as well as other cholangiopathies such as PBC. As a result of the very encouraging experimental data in preclinical (P)SC models, norUDCA has undergone further clinical development for PSC. Phase I clinical trials have been successfully completed and a multicenter European Phase II dose-finding trial testing norUDCA in PSC is ongoing.

Other interesting opportunities for targeted therapy in cholangiopathies are agents directed at the bile acid receptors TGR5 and FXR. TGR5 is a G-protein coupled bile acid receptor at a plasma membrane, while FXR is a nuclear hormone receptor, and both receptors are involved in the regulation of metabolism and inflammation through bile acids. Notably, some TGR5 polymorphisms have recently been associated with pathogenesis of PSC and ulcerative colitis. TGR5 and FXR are selective antagonists as well as dual TGR5/FXR ligands are now available; importantly neither UDCA nor norUDCA are FXR or TGR5 ligands. A 6α-ethyl derivative of CDCA (also known as 6-ECDCA or INT-747 or obeticholic acid [OCA]) had beneficial effects in mouse models of chemically-induced liver injury or in bile duct-ligation. In a phase II clinical trial in PBC patients not responding to UDCA, addition of OCA showed substantial reduction of biochemical cholestasis parameters and OCA monotherapy also improved biochemical cholestasis parameters. Dose dependent itching was the most common adverse event in patients receiving higher doses of OCA. A multicenter, placebo-controlled, randomized phase III clinical trial, testing OCA in PBC patients who have not non-responded to standard UDCA is about to be completed.

In the Mdr2 (Abcb4)−/− cholangiopathy model a dual ligand with high affinity to FXR (INT-767, but not the clinicial lead compound INT-747/OCA) was able to cure bile duct injury. Subsequent studies in FXR knock-out mice revealed that these effects were mediated exclusively by FXR and not by TGR5. The therapeutic mechanisms
involved suppression of bile acid synthesis and direct anti-inflammatory and antifibrotic effects and silencing of the reactive cholangiocyte phenotype. Notably, similar to norUDCA this therapeutic effect was also linked to generation of a bicarbonate-rich choleresis which appears to be a common denominator for successful treatment of cholangiopathies in general. In conclusion, the translation of expanding knowledge from animal experimental models should result in optimization of the currently available therapies for chronic inflammatory bile duct diseases such as PSC and PBC.
Pathophysiology of primary sclerosing cholangitis

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Despite the fact that immune mechanisms are involved in the development of primary sclerosing cholangitis (PSC) evidenced by T cell infiltrates upon histopathological examination its exact etiology and pathophysiology remain to represent a challenge for scientists and clinicians alike. PSC develops primarily in younger male subjects who for the most part also suffer from inflammatory bowel disease (IBD), namely ulcerative colitis (UC), or less frequently Crohn's disease (CD). In contrast to primary biliary cirrhosis (PBC), which affects females more often than males, is characterized by autoantibodies directed against specific mitochondrial autoantigens, and is associated with a variety of immune-mediated diseases, PSC only partly fulfils the general criteria of an autoimmune disease. There are no disease specific autoantibodies or defined autoantigens, immunosuppressants are ineffective as treatment modality, and unlike PBC and autoimmune hepatitis (AIH) PSC is associated with a considerable risk for both cholangiocellular (CCC) and colorectal adenocarcinoma (CRC). Based on this description the diagnosis of PSC is still today primarily based on the visual aspect of bile duct injury and not on a specific biochemical, immunological or functional test, which indicates the absence of a conclusive pathophysiological and all-encompassing etiological hypothesis.

In an attempt to search for clues for relevant pathophysiological features of PSC it is interesting to observe, that very different conditions can lead to a cholangiopathy, and specifically to sclerosing cholangitis. Diseases leading to a biliary pathology include ischemia-type biliary lesions following liver transplantation, secondary sclerosing cholangitis following intensive care therapy and septicemia, drug toxicity, genetic variations of bile transporter proteins (MDR3/mdr2, animal model), graft versus host disease, Alagille syndrome, biliary atresia, infectious cholangitis, Caroli syndrome, IgG4 disease, sarcoid disease, and cystic fibrosis. The range of these diseases indicates that the underlying pathophysiology spans a broad range of mechanisms. These cover areas as different as genetic diseases, control of bile composition and transport, infection, immune activation, tolerance, microcirculation and chemical toxicity. In light of the multitude of mechanistic processes that can lead to biliary injury PSC is either a disease end point with multiple subgroups of diseases, or a common to date elusive principle within all of these conditions exists capable of explaining all features of PSC.

To date most approaches to PSC have focussed on individual aspects of its pathophysiology. In general, it is likely that biliary epithelial cells (BEC) play a role in immune activation. In many diseases anti-BEC antibodies are detectable that can activate BEC to produce IL6 and CD44, activate ERK1/2 signalling, and upregulate toll-like receptors (TLR). TLR upregulation leads to the recruitment of immune cells and to inflammation. It is likely that this process can be activated by a multitude of stimuli, which may include systemic infection/septicemia, ischemia, altered bile composition and flow, and drug toxicity. The prominent association with ulcerative colitis has additionally led to the hypothesis of an enterohepatic immune axis. Evidence has been provided that homing of lymphocytes primed in the colon can proceed to the liver to induce portal field inflammation. However, PSC can exist in the
absence of UC and only few UC patients develop PSC. Moreover, patients after liver transplantation (LT) with an intact colon have an inferior course (more PSC recurrence) compared to those with a proctocolectomy prior to LT. Homing alone is therefore not capable of explaining all features. The best evidence for an immune-mediated mechanism yet is IgG4-associated cholangitis, which shows an infiltrate of IgG4 expressing B cells and a prompt response to immunosuppression with corticosteroids and/or azathioprine. While immunosuppression is not effective in all other cases of PSC IgG4 disease appears to be the result of an immune process, which can be interrupted by immune modulation.

A toxic model of sclerosing cholangitis has been developed by creating mdr2−/− mice, which exhibit a picture very similar to PSC but without co-existing IBD. In this scenario the lack of phospholipid in bile is the offending mechanism, a feature not present in humans suffering from PSC.

Genome wide association studies have highlighted the genetic risk for PSC and have identified HLA genes, genes involved in immune regulation and bile composition, as well as a number of non-HLA genes. To date, 16 main loci have been identified, which are capable of explaining 7.3% of variance in PSC liability. A difference exists between the closely related risk factors for UC. Overall, genetic data suggests that immune-mediated processes are among the greatest risk factors for PSC.

Unlike the other hepatic autoimmune diseases AIH and PBC, PSC is associated with a considerable risk for adenocarcinoma of the biliary system and the colorectum. In view of defining a specific pathophysiology for PSC it is interesting to acknowledge that UC in PSC is a different disease with more pancolitis, more right-sided colitis, rectal sparing, and back-wash ileitis, and leads to higher numbers of CRC than UC without PSC. The peak incidence of CRC is about 2 decades earlier in PSC patients than in the general population with a higher number of dysplasias and right-sided CRC. PSC is associated with a 10–20% risk of CCC but no known elevation of HCC risk.

The pathophysiology of PSC includes an immune-mediated component most likely regulated by genetic factors. This can proceed in the presence and the absence of IBD and either requires an internal stimulus or a putative external inducer. The resulting inflammation of the biliary system leads to fibrogenesis, stricturing and bacterial superinfection, which may also serve to increase the speed of biliary injury. Specific pathogens have not been convincingly identified to be responsible for PSC. Against the background of the multitude of conditions leading to sclerosing cholangitis, what we designate as PSC may well be a collecting pool of subentities with an array of specific etiologies that lead to a common end point – the macroductal destruction of the biliary tree.
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Pathology of bile duct carcinoma

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Bile duct carcinoma (cholangiocarcinoma) remains an intractable malignancy and is clinically detectable at the advanced stage in a majority of cases. Histologically, this carcinoma shows usually tubular and/micropapillary adenocarcinoma with variable fibrous stroma. Recently, two types of pre-neoplastic or early neoplastic lesions of bile duct carcinoma have been proposed in WHO 2010 tumor classification: biliary intraepithelial neoplasm (BilIN) and intraductal papillary neoplasm of bile duct (IPN-B). BilIN is a flat lesion and recognizable microscopically, and is graded into BilIN-1 (low grade), BilIN-2 (intermediate grade), and BilIN-3 (high grade) according to the atypia. BilIN is frequently found in the bile ducts around carcinoma and also in chronic biliary diseases such hepatolithiasis which is occasionally associated with cholangiocarcinoma. BilIN-3 is regarded to be followed by invasion of carcinoma into the surrounding tissue. IPN-B is a grossly visible intraductal papillary lesion in variably dilated bile duct lumen and is histologically graded into low, intermediate and high grade according to their atypia, and show four phenotypes (pancreatobiliary, gastric, intestinal and oncocytic). IPN-B of high grade is associated with an invasion into the surrounding tissue. IPN-B occur in an apparently normal bile duct and also in chronic biliary diseases such as hepatolithiasis and liver fluke infection. In this symposium, characteristic histological and immunohistochemical features and also molecular abnormalities of these lesions will be presented.
Session III

Targeted therapies in CCC
Current treatment of CCC – Where do we stand?

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Cholangiocarcinoma (CC) is the second most common primary liver cancer. The incidence in western countries increases and currently up to 1/100,000 people are diagnosed with CC per year. CC defines all tumors originating from gallway epithelium, including intrahepatic CC (ICC) and extrahepatic CC (ECC), as well as gallbladder carcinoma (GC). ECC can be divided into perihilar carcinoma and distal ECC. Radical resection is the only curative treatment option. However, high rates of irresectable patients and recurrence after resection result in overall poor prognosis. Because of the low prevalence and incidence of CC, conducting large prospective studies is difficult in CC. Only few randomized phase III-trials exist and so, many questions remain open in the treatment of CC, for example the role of adjuvant treatment or second line chemotherapy.

In cases with a potentially curative surgery, 5-year survival rates of 25–30% are reported. Currently, two studies have been initiated in Germany to determine the role of adjuvant therapy after surgery. The randomized cli ACTICCA study funded by the Deutsche Krebshilfe evaluates the role of adjuvant chemotherapy with gemcitabine and cisplatin compared to observation after curative intent resection of cholangiocarcinoma. The Product trail determines the feasibility and efficacy of adjuvant gemcitabine chemotherapy after liver transplantation for proximal bile duct cancer. This study will also provide important information about the role of liver transplantation for biliary cancer. Recent data from retrospective studies in the US indicate that liver transplantation may achieve excellent survival for patients with early-stage perihilar cholangiocarcinoma. In the palliative setting the intent of therapy is to extend life, relieve symptoms of obstructive jaundice and improve quality of life. Subclinical or frank cholangitis is associated with increased morbidity and mortality and endoscopic biliary drainage is an established procedure for palliation of unresectable malignant hilar biliary strictures. The role of intraductal radiofrequency ablation and photodynamic therapy, which consists of a photosensitizing agent in combination with laser irradiation, in selected patients is unclear and needs to be evaluated in prospective trails. In metastatic disease, chemotherapy improves quality of life and survival, and gemcitabine with cisplatin represents the standard of care. However, all patients ultimately progress on this therapy, so clinical trials of new and better agents are essential to expand the existing treatment options for patients with biliary cancer.
What can we learn from pancreatic cancer?

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The liver and the pancreas derive from the ventral endoderm of the foregut at almost the same time. Despite this close embryonic relationship, the liver and pancreas differ in their regenerative capacity. Specifically, the pancreas exhibits minimal regrowth following partial pancreatectomy, in contrast to the liver’s robust response following partial hepatectomy. Nevertheless, several parallels between regeneration in the liver and pancreas do exist. One similarity is that the nature of the regenerative response depends upon the nature of the injury. The cellular basis of normal tissue homeostasis in the liver and pancreas is the replication of existing cells to compensate for the low rate of cell death that occurs throughout life.

The close relationship between the biliary system and the pancreas is evident in the peribiliary glands. Yet it has proposed that the biliary tract is an incomplete pancreas. The intrahepatic large and extrahepatic bile ducts are accompanied by peribiliary glands. These glands drain into the bile duct and are positive for pancreatic exocrine enzymes and also lactoferrin and lysozyme. Exocrine acini of the pancreas are physiologically intermingled with peribiliary glands, though their distribution is usually patchy and infrequent. In addition, a-amylase and trypsin are also immunohistochemically observed in the lining epithelia of the intrahepatic large ducts and septal ducts.

There is plasticity to each other during development. Hes1, a Notch target blocks pancreatic differentiation of the biliary tract. Hes1 is expressed in the extrahepatic biliary epithelium throughout development. In Hes1 knock-out mice, biliary epithelium ectopically expresses the proendocrine gene Neurogenin 3, differentiates into pancreatic endocrine and exocrine cells and forms acini and islet-like structures in the mutant bile ducts, followed by the conversion of much of the extrahepatic biliary primordium to ectopic pancreas. Thus, biliary epithelium has the potential for pancreatic differentiation and may be involved in the pathogenesis of several biliary tract pathologies, particularly under neoplastic conditions with genetic and epigenetic alterations.

Most biliary and pancreatic neoplasia are of ductal lineage characterized by tubular, cystic, and/or papillary formations. The lesions are positives for mucin production and expression of mucin-related glycoproteins. The in situ spectrum of the ducts characterizing intraepithelial neoplasms, intraductal papillary neoplasms and mucinous cystic neoplasms shares similarities in both organs. Cholangiocarcinoma and invasive pancreatic ductal adenocarcinoma usually display abundant fibrosis, show frequent lymphatic and perineural invasion and are characterized by rapid dissemination to lymph nodes. Both are resistant to treatment approaches and have a poor prognosis suggesting that the biological behaviors of cholangiocarcinoma and ductal pancreatic adenocarcinoma are similar or close to each other. Biliary intraepithelial neoplasia (BiiLN) are now accepted as precursor lesions of cholangiocarcinoma similar to pancreas intraepithelial pancreatic neoplasms (PanIN) as precursors of pancreatic cancer. BiiLN and PanIN show similar histologies and display similar expression patterns of mucins and cytokeratins. In the pancreas Kras
activation in mature acinar cells induces PanIN lesions and Notch promotes this process. The exocrine acini in the peribiliary glands and biliary epithelia expressing pancreatic enzymes might be a source for BillN development. Interestingly novel data support the concept of hepatocytes as the cells of origin of cholangiocarcinoma. Similar to the pancreas, activated Notch2 converts adult hepatocytes to the biliary lineage. Thus conversion of pancreatic acinar cell as well as hepatocytes appears to be an initial step in the carcinogenesis to pancreatic cancer and cholangiocarcinoma.
New treatment strategies for CCC – What can we hope for?

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Cholangiocellular carcinomas (CCC) are increasing worldwide, but the causes of this global increase are not clear yet. Known risk factors include biliary diseases such as primary sclerosing cholangitis (PSC) and parasitic infestation of the biliary tree, but most cases are not associated with any of these underlying diseases. So far surgical approaches provide the only curative modalities. Due to the limited knowledge of CCC pathogenesis, only a few molecular therapies have been tested successfully. Several signaling pathways like VEGF, EGF, KRAS, MAPK and STAT have been found to be deregulated in CCC. We have investigated the involvement of Hedgehog in epithelial to mesenchymal transition (EMT), migration and CCC tumor growth. Cyclopamine application inhibited successfully cell proliferation, migration and invasion by down-regulating Hedgehog target genes, glioblastoma 1 (Gli1) and glioblastoma 2 (Gli2). In vivo therapy inhibited the growth of CCC Xenograft tumors. We have also shown that Notch inhibition by γ-secretase inhibitor IX (GSI IX) impairs cell viability, migration, invasion, EMT and growth of softagar colonies. In vivo overexpression of Notch-ICD together with an inactivation of p53 significantly increased tumor burden and showed CCC characteristics. However, a multi-disciplinary approach that brings together biologists, clinicians and pharmacologists is still required to fight against this insidious disease.
Session IV

Interventional treatment of CCC
Biliary endoscopy – Molecular diagnosis and treatment options

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Biliary endoscopy has significantly impacted on our approach to intraductal pathologies. Cholangioscopes have become smaller as resolution has increased at the same time. Whereas cholangioscopy via mother-baby-endoscopes used to be an examination requiring two examiners, newer systems now use single operator cholangioscopy via a duodenoscope or direct peroral cholangioscopy. Clinical trials have mostly examined cholangioscopy for indeterminate biliary strictures which often pose a clinical dilemma. Conventional methods to evaluate strictures include brush cytology and biopsy and have an excellent specificity, but sensitivities well below 50%. Diagnostic outcome is optimized when direct visualization is combined with cholangioscopy-guided biopsies, but results remain less reliable than in other endoscopic fields. The availability of probe-based endomicroscopy has permitted transpapillary intrabiliary microscopic imaging. While this yields microscopic details at real time, it relies on systemic administration of an unspecific fluorescent dye. Molecular imaging uses targets on tumor cells for specific imaging, comparable to immunohistochemistry. At present, molecular imaging in endoscopy has two aims, enhanced detection and lower miss rate of lesions and improved characterization that can even be used for prediction of response to molecular targeted therapy. These applications are still under investigation, but hold a promise for individualizing targeted therapy in the future.

In addition to improving diagnosis, cholangioscopy is also used to plan therapy in clinical routine. It allows visual guidance and planning of stenting, but also of radio frequency ablation or photodynamic therapy for malignant strictures. Laser lithotripsy of biliary stones is another well established indication. In summary, advances in biliary endoscopic techniques - although still not reaching the accuracy of other endoscopic procedures - have improved our understanding and access to pathologies of the bile ducts and thereby the diagnostic yield and endoscopic work up for these patients.

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Surgical treatment of CCC – What are the options?

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Bile duct carcinomas (cholangiocarcinomas, CCC) are a rare, but highly fatal disease. They are classified in intrahepatic, hilar and distal extrahepatic bile duct tumors according to their localization in the biliary system. The majority of cases are extrahepatic hilar cholangiocarcinomas originating from the hepatic bifurcation (Klatskin tumor). Cholangiocarcinomas are relatively uncommon and account for only 3% of all gastrointestinal cancers. Surgical resection represents the only potentially curative treatment option for cholangiocarcinomas. The extent of the surgery depends on the site of the cancer within the liver and/or the biliary tract. For locally contained disease, resectability is primarily determined by the extent of biliary or vascular involvement. Resection includes the involved segments together with a regional lymph node dissection. In most cases of intrahepatic and hilar cholangiocarcinomas extended resections are necessary to achieve R0 resection. Especially in hilar cholangiocarcinomas surgical management is highly complex. Major problem of extensive liver surgery in hilar cholangiocarcinoma is an increased perioperative morbidity and the risk of postoperative liver dysfunction. Therefore an optimal preoperative conditioning of the future liver remnant is therefore mandatory. Almost half of the patients with resectable intra- or extrahepatic bile duct cancer have regional lymph node involvement at the time of diagnosis. However, this is not a contraindication for surgery unless para-aortal nodes are involved. Curative treatment by radical surgical procedures with surgical preparation distant to the tumor region results in 5-year survival rates of 25–50%, depending on patient selection and tumor stage.

In a selected population of patients with small, irresectable hilar cholangiocarcinomas liver transplantation might be an option. These include PSC patients with hepatic dysfunction or locally unresectable tumors in an otherwise normal liver. Recent data using strict patient selection and adjuvant or neoadjuvant protocols have shown encouraging results. Using strict selection criteria including small tumor size (< 3 cm) and negative lymph nodes, the Mayo Clinic protocol of neoadjuvant chemoradiation, followed by liver transplantation has achieved a 5 year recurrence free survival of 65% in a recent multicenter analysis. At present liver transplantation does not represent a standard treatment for these patients, but it can be considered for carefully selected patients with hilar cholangiocarcinomas.
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POSTER ABSTRACTS

Poster Numbers 1 – 34

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Expression of hypoxia inducible factors in a rat model of thioacetamide (TAA)-induced chronic liver damage and cholangiocarcinoma

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Introduction: Hypoxia has been shown to have a role in the pathogenesis of several forms of liver disease. The Hypoxia Inducible Factors (HIFs) are a family of evolutionarily conserved transcriptional regulators that affect a homeostatic response to low oxygen tension and have been identified as key mediators of angiogenesis, inflammation, and metabolism.

Methods: Male wister rats were administrated thioacetamide (TAA) in drinking water up to 20 weeks and sacrificed at different time points (4, 8, 12, 16, 18, 20 weeks). Expression of HIF-1α and HIF-2α was evaluated during chronic liver damage and cholangiocarcinoma. Expression of HIF-1α and HIF-2α was measured by RT-PCR and Western blot. HIF-1α, HIF-2α, α-SMA, CK-19, ED1 and ED2 were localized by immunofluorescence staining.

Results: HIF-1α was colocalized with α-SMA, during 8 and 16 weeks of hepatic damage suggesting its role in liver fibrogenesis. We have also seen HIF-1α in CK-19 positive tumour areas and regenerating nodules (18w and 20w). HIF-2α appeared in α-SMA positive-cells during 16w whereas tumour areas were negative for HIF-2α. However, HIF-2α was localized in the endothelial cells and sinusoids, showed positivity in ED1+ cells during 8w while HIF-1α positivity in infiltrating ED1+ cells was detected in chronically damage-liver and cholangiocarcinoma. The immunofluorescence analysis revealed HIF-2α positivity and HIF-1α negativity in ED2+ cells. RT-PCR and Western Blot data confirmed the immunohistochemical analysis.

Discussion/Conclusion: HIF-1α expression in chronically damaged-liver and tumour areas clearly describes its role as damage-associated molecule during hepatic repair and fibrogenesis. The detection of HIF-2α in endothelial-cells indicates its role in the recovery-phase of chronic liver-damage, angiogenesis and vascularisation. The expression of HIF-1α in cholangiocarcinoma suggests a close relationship to tumour angiogenesis and infiltration. The overexpression of HIF-1α can be used as important indices to evaluate biological behaviours. HIF-1α may become a new target for cholangiocarcinoma prognosis and treatment.
Age-related changes in the antioxidant system of the liver

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Introduction: One hypothesis states that aging is caused by the accumulation of free radical production caused by oxidative stress. Changes in the antioxidant system are expected to be partially responsible for increased oxidative stress during aging. In this study, a rat model was used to examine whether the antioxidant system of the liver is affected by aging.

Methods: The glutathione (GSH) system, along with the corresponding enzymes, such as superoxide dismutase (SOD), Catalase (Cat), GSH Peroxidase (GPx) and GSH reductase (GR), were investigated regarding their activity and on protein level in liver tissue of young (6 weeks), middle-aged (6–7 months) and old (23 months) rats. For basal values, livers of untreated rats were used. For oxidative stress induction, rats were treated in heat cabinets (body temp. > 40°C for 30 min on two subsequent days) and were sacrificed afterwards. Liver tissue and serum were collected at different time points.

Results: Several alterations of basal antioxidant enzyme activities and of the GSH system of the liver during aging were observed. The total GSH content was significantly increased in old rats. On the enzyme activity level, GPx and SOD were increased, while GR was lower in the old group compared to middle-aged rats and Cat was not affected by aging. After heat stress, differences in the stress response could be observed, indicating that old rats have a more oxidized hepatic environment and are not able to cope with oxidative stress to the same extent than middle-aged rats. As an example, up-regulation of GPx was much slower in old rats.

Conclusion: Our results suggest that the organism of the elderly disposes only of a restricted compensation of oxidative stress caused by several diseases. Regarding the liver, these observations could be critical regarding the recovery from hepatic resections or NAFLD.

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Evaluation of a chip-based 3D culture system for the use of primary human hepatocytes in drug toxicity testing

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Introduction: To date, toxicity studies in the preclinical phase of drug development are mainly done in vivo with rodents. However, as the predictability for humans is limited and large numbers of animals are needed, there is rising attempt to develop alternative in vitro systems. However, most systems poorly reflect the in vivo situation, as static monolayer cultures of hepatocytes rapidly lose their functions such as urea production, cytochrome P450 and transporter activities. 3D culture under continuous flow has been shown to significantly improve these functions. In the present study, the suitability of a chip-based miniaturized bioreactor (r-3D-KITChip) for culture of primary human hepatocytes and its application for in vitro toxicity testing has been investigated.

Methods: Primary hepatocytes were isolated from liver resectates by a two-step collagenase perfusion procedure. Experiments were in accordance with the ethical standards of the responsible committee on human experimentation. Hepatocyte functionality was measured by different photometric and fluorometric methods. Acetaminophen-induced toxicity was determined by a colorimetric viability assay and by measurement of lactate dehydrogenase leakage after a 24-h treatment period.

Results: Primary human hepatocytes cultured under continuous flow in the r-3D-KITChip bioreactor were shown to significantly improve hepatic functions such as urea production and hepatic transporter activity compared to static monolayer culture. Furthermore, hepatocytes showed a higher sensitivity towards acetaminophen in the r-3D-KITChip bioreactor.

Conclusion: Taken together, the r-3D-KITChip bioreactor appears to be suitable for the maintenance of hepatic functions of primary hepatocytes in culture for up to 72 h. It thus can be used for acute toxicity testing in vitro. We are currently extending the culture time up to two weeks in order to evaluate the suitability of this culture system for hepatotoxicity testing after long-term exposure.

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Hypoxia influences expression of genes involved in angiogenesis and EMT in HCC cells

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Introduction: Hepatocellular carcinoma (HCC) is the fifth most frequent cancer worldwide and its incidence in Western countries has significantly increased in the last decades. In most cases, HCC-patients have a background of chronic liver disease leading to liver cirrhosis, which is the main risk factor for the development of HCC. In most cases, HCC is diagnosed at an advanced stage and surgical resection is often impossible in cirrhotic tissue. Therefore, therapies such as transarterial (chemo)embolization (TACE) are of great importance. However, TACE leads to hypoxia, which has been associated with induction of a more aggressive phenotype in remaining HCC.

The aim of this study was to analyze the effects of hypoxia on expression of genes relevant for tumorigenicity and therapy resistance in HCC.

Methods: HepG2 HCC cells were maintained in normoxic or hypoxic (1% O₂) conditions for 48 h. Subsequently, RNA was isolated and transcribed into cDNA, and gene expression analysis was performed using quantitative real-time PCR.

Results: We found significantly increased expression of Interleukin-8 (IL-8) in hypoxic cells compared to normoxic cells. Increased expression of IL-8 is known to induce angiogenesis in HCC. Furthermore, we observed significantly increased expression of SLUG under hypoxic conditions. SLUG is an established maker of epithelial-mesenchymal transition (EMT), which is known to correlate with enhanced invasion and metastasis of HCC cells. Furthermore, we analyzed genes responsible for drug resistance. ABC-transporter ABCC1 (MRP-1) was significantly upregulated in hypoxic cells, though expression of ABCB1 (MDR-1) remained without significant changes.

Conclusion: Our results indicate that hypoxic conditions in HCC, as for example induced by transarterial (chemo)embolization (TACE), enhance angiogenesis and EMT in HCC cells. Furthermore, hypoxia induced enhanced expression of a key gene responsible for chemotherapy resistance in HCC. Together, these findings indicate that TACE may be a two-edged sword, and suggest that TACE should be combined with therapeutic strategies counteracting hypoxia-mediated signaling mechanisms which favor aggressive growth of remaining cancer cells.
Monoallelic *MUTYH* hotspot mutations do not confer substantial genetic susceptibility to cholangiocarcinoma

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**Background and aims:** Inflammation-associated oxidative stress and DNA damage are involved in malignant transformation of cholangiocytes. Defective DNA repair mechanisms might predispose to cholangiocarcinoma (CCA). Since the role of *MUTYH* germline mutations remains controversial, the aim of this study was to re-evaluate the *MUTYH* hotspot mutations p.Y179C (rs34612342) and p.G396D (rs36053993) as genetic susceptibility factors in the largest CCA cohort genotyped so far.

**Patients and methods:** The study population consisted of 222 European CCA patients (65.9 ± 11.8 years, 133 males, 89 females; 45 intrahepatic, 177 extra-hepatic; tissue diagnosis in 77.9%) and 355 controls (61.0 ± 11.0 years; 158 males, 197 females). *MUTYH* hotspot variants were genotyped using TaqMan assays.

**Results:** Monoallelic hotspot mutations were detected in 4 patients (3 p.G396D; 1 p.Y179C) whereas 6 control subjects were heterozygotes (5 p.G396D; 1 p.Y179C). None of the patients was a biallelic hotspot mutation carrier. The observed allele frequencies did not differ significantly between cases and controls (p > 0.05) and association tests did not provide evidence for an involvement of p.Y179C (OR 1.6 [95% CI: 0.1–26.0]) or p.G396D (OR 1.0 [95% CI: 0.2–4.0]) in CCA susceptibility. However, given the very low minor allele frequency, power analysis identified a sufficient power only for large effect sizes (96% for an OR of 6.5 for p.G396D and 78% for an OR of 14.0 for p.Y179C).

**Conclusions:** Monoallelic *MUTYH* hotspot mutations do not represent genetic susceptibility factors conferring substantial CCA risk in Caucasians. Thus, our results argue against routine *MUTYH* mutation testing to identify patients at risk for developing CCA. Due to the low statistical power for the identification of smaller allele effects much larger studies are needed to detect effects with marginal clinical significance. However, the CCA risk for patients with biallelic mutations und *MUTYH*-associated polyposis should be refined in future work.
Upregulation of SPRED and SPROUTY2 in the pathogenesis of chronic liver disease

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Introduction: Hepatocellular carcinoma (HCC) mostly develops in patients with chronic liver disease and liver cirrhosis is the major risk factor for the development of HCC. Non-alcoholic fatty liver disease (NAFLD) is recognized as the most frequent liver disease in Western countries and its incidence is still rising. It is characterized by hepatic lipid accumulation which starts with simple hepatic steatosis and progresses towards hepatocellular injury and inflammation (non-alcoholic steatohepatitis [NASH]) in a significant number of patients. In addition, there is increasing evidence that liver steatosis is a risk factor for HCC development and progression.

The mammalian SPROUTY family consists of 4 members of signal transduction proteins, which were shown to be involved in pathways affecting cancer initiation and progression such as angiogenesis, cell growth, migration, invasion and cytokines. SPROUTY2, an inhibitor of Ras/ERK pathway and fibroblast growth factor (FGF) signaling was shown to be downregulated in HCC and its overexpression had a tumor suppressive effect. The SPROUTY-related enabled/vasodilator-stimulated phosphoprotein homology 1 domain-containing protein (SPRED) was also shown to inhibit Ras/ERK signal transduction. Reduced SPRED expression was published for HCC and this resulted in a more malignant phenotype. Despite its role in HCC development and progression the expression of these negative signaling regulators in chronic liver disease including NAFLD has been unknown.

The aim of this study was to analyze the expression of SPROUTY2 and SPRED in in vitro models of hepatic steatosis and in in vivo NASH-models.

Methods and results: Primary human hepatocytes (PHHs) were stimulated with free fatty acids (FFA) palmitate and oleate which led to a dose dependent induction of cellular lipid accumulation. Quantitative RT-PCR revealed increased hepatic SPROUTY2 and SPRED mRNA expression in lipid loaded PHHs compared to control cells. Next, we analyzed SPROUTY2 and SPRED expression in mice fed with a NASH inducing diet which induces significant hepatic steatosis and inflammation which closely mimics the pathology observed in NASH patients (Matsuzawa N et al. Hepatology. 2007; Dorn C et al. Mol Nutr Food Res. 2010). Both SPROUTY2 and SPRED mRNA expression were significantly higher in mice exposed to the NASH inducing diet compared to mice fed with a standard chow. We also analyzed mice fed with a high fat diet which causes significant hepatic steatosis but only minimal hepatic inflammation. Notably, also in this model we observed increased expression of SPROUTY2 and SPRED mRNA compared to control mice.

Conclusion: Together, these data indicate that even in the absence of significant inflammation hepatocellular lipid accumulation leads to a significant induction of SPROUTY2 and SPRED expression in the liver. Considering the known protumorigenic effect of reduced expression of these two negative regulators of
signal transduction pathways in HCC, these data are surprising and raise the question at what stage of HCC development and progression the downregulation of SPROUTY2 and SPRED occurs. The underlying mechanisms of this switch in expression may be promising diagnostic and therapeutic targets in HCC.
Y-box-binding protein 1 is a critical mediator in cholangiocarcinoma

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Introduction: Y-box-binding protein 1 (YB-1), a prototypic member of cold shock protein family, is carcinogenic in different organs. The present study investigated the role YB-1 in cholangiocarcinoma (CCA), which has not been assessed so far.

Methods: Twenty-nine patients with CCA were enrolled in this study. Immunoblotting and immunohistochemistry (IHC) were performed to measure levels of YB-1 in liver tissue from patients. The correlation between IHC immune score and clinical characteristics of all enrolled CCA patients were calculated. In vitro, biological effects of YB-1 were measured in four cholangiocarcinoma cell lines (HCCC9810, HuH28, TKF-1 and Zo).

Results: Immunoblotting and IHC showed markedly elevated YB-1 expression in cancer tissues as compared to surrounding non-cancer tissues of CCA patients. YB-1-positive staining localized in nuclei and/or cytoplasm of cancer cells. Cytoplasmic YB-1-positive cancer cells correlated with cholestasis in CCA patients (p < 0.05). Nuclear YB-1-positive cancer cells significantly correlated with tumor size, vascular invasion and clinical stages of CCA (p < 0.05 for each). Follow-up study showed that patients with YB-1-positive nuclear staining in cancer cells had significant shorter overall and disease free survival time. In vitro, MTT analysis indicated that knockdown of YB-1 expression with RNAi inhibited proliferation of cancer cells in four CCA cell lines tested. Further, growth inhibition of HCCC9810 and Zo cells by Gemcitabine was partially blocked after these cells were transfected with an YFP-YB-1-CFP expression plasmid and ectopic expression of YB-1.

Discussion/Conclusion: YB-1 contributes to progression of CCA at least through promoting proliferation of cancer cells. This transcription factor may render CCA cells resistant for chemotherapeutic treatment.
Sustained virological response is low in diabetic Egyptian chronic hepatitis C patients and zero % in patients who developed DM during Peg interferon/ribavirin therapy

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**Introduction**: (DM) is common in (CHC) patients and reduces the therapeutic effectiveness of pegylated interferon (peg IFN), ribavirin therapy.

**Aims**: To study the effect of DM on the sustained virological response (SVR) after peg IFN, ribavirin in Egyptian CHC patients and the incidence of glucose abnormalities developed in non diabetic patients and its relation to the SVR.

**Methods**: 256 Egyptian CHC patients were divided into Group (1): 116 diabetic patients, and Group (2): 140 non-diabetic patients. All received Peg IFN and ribavirin for 48 weeks and monitored for SVR and glucose abnormalities.

**Results**: SVR of the study group was 50.8% (40.50% for diabetics and 59.30% for non-diabetics, p = 0.003), on multivariate analysis, diabetic patients were significantly older [OR = 1.06, 95% CI: 1.016–1.114, p 0.008], had higher BMI (OR = 1.204, 95% CI: 1.020–1.422, p = 0.03) and steatosis (OR = 1.565 95% CI: 1.21–2.20, p = 0.001). Low SVR was associated with higher fasting and post prandial blood sugar (p < 0.05), steatosis (p = 0.04), grade 3 fibrosis (p = 0.039) and high viral load (p = 0.001). During treatment of non-diabetic CHC patients, glucose abnormalities developed in 25 patients (18%), they have less SVR (36% vs. 64.3%, p = 0.009), older age (p = 0.001), higher BMI (p = 0.001) and higher fibrosis F3 (p = 0.001), 7 patients (5%) developed DM and their SVR was zero %.

**Discussion/Conclusion**: DM significantly decreases the SVR in Egyptian CHC patients (40.5% in diabetic and 59.3% in non diabetic patients). In non-diabetics, 5% of patients developed DM and had zero % SVR.
BRAF V600E specific immunohistochemistry reveals low mutation rates in biliary tract cancer and restriction to intrahepatic cholangiocarcinoma

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Introduction: BRAF mutations have emerged as an important predictive biomarker for metastasized melanoma. Other types of cancer may also benefit from BRAF mutation targeted therapies. In biliary tract cancer, reported BRAF mutation rates are highly controversial, ranging from 0% to 33% in adenocarcinoma of the gallbladder and 0% to 22% in cholangiocarcinoma.

Methods: We here analyzed tissue microarrays of a large cohort of biliary tract cancer (n = 377) including 159 intrahepatic cholangiocarcinomas, 149 extrahepatic cholangiocarcinomas, and 69 adenocarcinomas of the gallbladder for BRAF V600E mutation using a highly sensitive immunohistochemical screening approach implementing the BRAF V600E protein specific antibody VE1. All VE1 positive cases as well as 42 VE1 negative cases were additionally analyzed by Sanger sequencing.

Results: In total, only five VE1 positive cases were detected (5/377; 1%). BRAF V600E mutation was confirmed by direct sequencing in all cases. All 5 mutated cases were intrahepatic cholangiocarcinomas (5/159; 3%). None of the extrahepatic cholangiocarcinomas and adenocarcinomas of the gallbladder were VE1 positive. Apart from the subtype-restriction of BRAF V600E mutation to intrahepatic cholangiocarcinoma and a female predominance (4 female, 1 male), no significant correlation with clinicopathological data and patient outcome was detected.

Discussion/Conclusion: In conclusion, we demonstrate that BRAF V600E mutation is a rare event in biliary tract cancer accounting for only 1% of all subtypes and is restricted to intrahepatic cholangiocarcinoma. Additionally, we demonstrate that VE1 immunohistochemistry is a feasible approach to routinely screen for BRAF V600E mutation in biliary tract cancer patients thereby facilitating the detection of rare patients that may benefit from BRAF mutation targeted therapies.
Expression pattern of epithelial-mesenchymal transition-associated miRNAs in biliary tract cancer: A clinical and pathological approach

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Introduction: Epithelial-mesenchymal-transition (EMT) is a key mechanism in tumor progression and metastasis which is regulated among others via microRNA-200 (miRNA) family members. Referring to this, we investigated these six miRNAs and their expression pattern in cancer of the biliary tract (BTC) to evaluate their possible role in tumor progression and (de-)differentiation.

Methods: Total RNA was isolated from FFPE blocks (n = 34) obtained from surgically resected BTC patients between 1997 and 2013 using the miRNeasy FFPE isolation kit (Qiagen®). Expression levels of six miR-200 family members (miRNA-141, -205, -429, -200a, -200b, -200c) were measured by quantitative RT-PCR followed by statistical analyses to investigate possible relationship between miRNA expression and clinical-pathological data. Additionally, tissue microarrays of these BTCs were characterized for proliferation (p16, p27, p53 and Ki-67) and differentiation markers (Vimentin, Cytokeratin-7 and -19) by immunohistochemistry.

Results: Overall, we observed a collinear expression pattern of all six miRNAs in BTC tumor specimen’s independent from tumor grade or stage. Interestingly, once lymph node or other metastatic dissemination occurred, all miRNAs were significantly down-regulated in these advanced tumor stages, suggesting a threshold of miRNA expression levels and tumor spread. Additionally, tumor specimen with high Vimentin protein expression pattern (indicating mesenchymal differentiation) revealed a significant association with reduced miRNA expression regarding tumor region analysis.

Discussion/Conclusion: We observed a highly coordinated and tumor stage-dependent as well as Vimentin-associated expression pattern of six miRNAs in BTC specimens. Their changes of expression in advanced tumor stages may indicate their possible key regulatory role in preventing tumor progression (EMT). Thus, finding the triggers for their down-regulation would provide possible clinical targets for early diagnosis of progression and therapy, respectively.

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Establishment of a human in vitro model to evaluate the immune response of Kupffer cells after hepatic tissue damage

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Introduction: Hepatic tissue damage can occur due to several diseases like infections or liver tumors. Kupffer cells (KC) play an important role in mediating tissue damage as well as in regenerative processes. KC activation by antigens or endogenous proteins can lead to the release of different cytokine patterns that support inflammation or induce tolerance reactions. Aim of the present study was the establishment of a human in vitro model which enables the investigation of immune mediated signaling after hepatocyte damage.

Methods: Primary human hepatocytes (PHH) and KC were isolated from human liver resectates using a two-step collagenase perfusion technique. KC activation was investigated by measuring intracellular formation of reactive oxygen intermediates (ROI) using the DCF assay. Mitochondrial activity as a marker for cell activity was measured using the XTT assay. In order to simulate the activation of KC following hepatocyte damage, KC were incubated with supernatants of PHH treated with hepatotoxic compounds. KC reaction was determined by measurement of cytokines using specific cytokine ELISA-kits.

Results: The isolated KC yield was 1.25 ± 0.89 x 10⁶ cells/g liver tissue with a purity of > 80%. Initial ROI levels in KC showed high donor variability, depending on the condition of the tissue and patient anamnesis. Incubation of KC with supernatants from compound-treated PHH increased mitochondrial activity and lead to a change in the formation of ROI compared to KC incubated with supernatants of untreated PHH. Additionally we detected a compound and donor dependent increase in proinflammatory cytokines TNF-α and IL-6 or in anti-inflammatory cytokines PGE₂ and IL-10, respectively.

Discussion/Conclusion: Treatment of KC with supernatants of stressed and/or damaged PHH lead to a donor and compound dependent KC activation linked with an immune response transmitted via cytokine production. Interpretation of the cytokine profile revealed that the KC reaction depends strongly on the donor anamneses and patients state of disease.
Effects of glucose levels on lipid metabolisms in hepatocellular carcinoma cells *in vitro*

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**Introduction:** There is emerging evidence that insulin resistance and high blood glucose levels promote cancer development and progression. It is also known that tumor cells gain a survival/growth advantage by adapting their metabolism to respond to environmental stress, a process known as metabolic transformation. Increased glucose and lipid uptake as well as *de novo* lipogenesis are believed to be involved in these processes.  
**The aim of this study** was to investigate the effects of glucose levels on the expression of regulators of hepatic lipid metabolisms in hepatocellular carcinoma (HCC).

**Methods:** Hep3B HCC cells were cultivated for 16 weeks in either high glucose (450 mg/dl glucose – “Hep3B high”) or low glucose (100 mg/dl glucose – “Hep3B low”) medium, mimicking diabetic and normal metabolic conditions, respectively. Quantitative RT-PCR analysis was performed to analyze the expression of key enzymes of cellular lipid metabolism.

**Results:** Hep3B high cells showed significantly increased expression of fatty acid binding protein 1 (FABP-1) compared to Hep3B low cells, indicating an increased uptake of fatty acids. Also expression of monoacylglycerol lipase (MGLL), catalysing the hydrolyzation of triglycerides into fatty acids, was higher in Hep3B high cells. The expression of other enzymes involved in beta-oxidation, like long-chain acyl-CoA dehydrogenase 1 (ACADL1), was also upregulated, while there was only a small increase in expression of carnitine palmitoyltransferase 1 (CPT1) and peroxisomal acyl-CoA oxidase 1 (ACOX1). There was no difference in acetyl-CoA carboxylase 2 (ACC2) expression, responsible for acetyl-CoA utilization in the citrate cycle, but increased expression of fatty acid synthase (FASN), a key regulator of *de novo* lipogenesis, and peroxisome proliferator-activated receptor gamma (PPAR-gamma), a transcription factor also involved in lipogenesis and lipid storage, as well as diacylglycerol acyltransferases 1 and 2 (DGAT1, DGAT2), the last enzymes of triglyceride assembly.

**Discussion/Conclusion:** Our data suggest that high blood glucose levels cause significant alterations in HCC lipid metabolisms, indicative of increased lipid utilization rather for *de novo* lipogenesis and proliferation than generation of energy through the citrate cycle, thereby promoting tumorigenity of HCC. Consequently, adequate anti-diabetic treatment appears to be important for HCC prevention and treatment.
Metal stent placement at ERCP in a DGH – Patient selection according to prognosis

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Introduction: ERCP is the established modality for palliative stenting and symptomatic relief of malignant biliary obstruction. Long term patency is better with metal stents and these are now recommended when the prognosis in malignant disease is greater than a few months to avoid the need for repeat procedures. The aim of this study is to evaluate survival and patient outcomes following metal stent placement in a District General Hospital linked to a regional cancer centre.

Methods: We analysed the data in Maidstone Hospital from 2007–2013 where our policy has been to place metal stents if the Multidisciplinary Group judged that the prognosis of the patient was greater than three months. Demographic data, need for re-intervention, survival post procedure and resolution of biliary obstruction were evaluated.

Results: 30 patients had metal stents placed during the 6 year period. The diagnosis was pancreatic cancer in 20 (67%), cholangiocarcinoma in 7 (24%), metastatic disease in 1 (3%), duodenal cancer in 1 (3%) and oesophageal cancer in 1 (3%). 1 patient (3%) needed re-intervention. 20 patients (67%) had good resolution of bilirubin levels following the procedure whereas 5 patients (16%) did not have resolution and the other 5 patients (16%) did not require blood tests following the procedure. One patient died from a septicaemia within 7 days (Cl perfringens) and at thirty days there were 7 (23%) deaths. The 3 month mortality was 57% (17 patients).

Discussion/Conclusion: All cases were technically successful with a very low re-intervention rate. Apart from the one patient who died of septicaemia, which was possibly procedure related, there were no deaths/morbidity related to the procedure. However, the significant death rate from underlying disease progression by 3 months (57%) suggests that our selection of patients for metal stenting is flawed and the Multidisciplinary Group is unduly optimistic when judging the overall prognosis of individual patients.
Analysis of natural killer cell receptor G2D (NKG2D) variant in a cohort of European patients with cholangiocarcinoma

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Introduction: Previously it was shown that genetic variants within the NKG2D-MICA pathway modulate the risk to develop cholangiocarcinoma (CCA) in PSC patients¹. Here we investigate the effects of the NKG2D variant on the prevalence of the CCA in a cohort encompassing mostly individuals with sporadic carcinoma.

Methods: Overall, we genotyped 221 patients with CCA (131 males; age 28–90 years; PSC prevalence < 2%) from Germany (n = 164) and Romania (n = 57). The control group consisted of 297 CCA-free individuals (131 males, age 22–90 years). The NKG2D single nucleotide polymorphism (SNP) rs261716¹ was genotyped using a PCR-based assay with 5'-nuclease and fluorescence detection. The association of the SNP with CCA was tested in contingency tables (chi² test for alleles; Armitage's trend test for genotypes).

Results: The association tests do not provide evidence for genetic risk modulation by the NKG2D variant either in the whole cohort (common OR = 1.207, p = 0.20) or after exclusion of individuals with PSC (common OR = 1.209, p = 0.20). Of note, in the analysis performed separately for individuals with extra- and intrahepatic tumor localization, we identified a potential association of this variant with intrahepatic tumors (n = 45, common OR = 1.631, p = 0.04). In contrast, we did not detect any association of the NKD2D polymorphism with extrahepatic CCA (n = 176, common OR = 1.108, p > 0.05).

Discussion/Conclusion: The NKD2D variant, previously associated with the risk of CCA in patients with PSC, might modulate the risk of intrahepatic bile duct cancer in individuals without PSC. These results, together with the previously published association¹, point to a potential genotype-based screening strategy for individuals who are at-risk of cholangiocarcinoma due to variant NKD2D.

Reference:
Decreased Smad3 phosphorylation at linker serine 213 is associated with poor prognosis of patients with intrahepatic cholangiocarcinoma

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Introduction: Differential phosphorylation of Smad3 at C-terminal and linker site has been shown to convey different roles in carcinogenesis. The present study investigated the association between phosphorylation of Smad3 at C-terminal/linker sites and clinical characteristics of intrahepatic cholangiocarcinoma (IHCCA).

Methods: Immunohistochemistry (IHC) was performed to detect the levels of TGF-β, p-Smad3C, p-Smad3L (ser204, ser208 and ser213) and cytokeratin19 (CK19) in 50 specimens from 25 IHCCA, 20 cirrhotic liver and 5 hepatic hemangioma. The correlation between TGF-β/Smad3C IHC score and clinicopathological characteristics of IHCCA was evaluated.

Results: p-Smad3L (ser213) was detected predominantly in normal bile duct epithelial cells (BDECs) in normal livers but also hepatic progenitor cells and reactive ducts in cirrhotic livers. Levels of p-Smad3L (ser213) were significantly reduced in cancer cells compared to surrounding non-tumor tissues. Well-differentiated IHCCA showed strong p-Smad3L (ser213) expression, whereas it was significantly reduced in cancer cells moderate- or poorly-differentiated IHCCA. Low expression of p-Smad3L (ser213) was associated with the presence of metastasis within 6 months and 12 months (p < 0.05). IHCCA patients with high levels of p-Smad3L (ser213) showed increased survival compared to those with low levels (p < 0.05). In contrast to p-Smad3L (ser213), elevated levels of p-Smad3C and TGF-β in IHCCA cancer areas were higher compared to surrounding non-tumor tissue. No significant correlation was shown between p-Smad3C and clinical characteristics.

Discussion/Conclusion: These data suggest that Smad3 differential phosphorylation is associated with tumor differentiation and or progression. Decreased p-Smad3L (ser213) indicates a poor prognosis for patients with IHCCA. Smad3 phosphorylation at linker serine 213 might play an important role in the homeostasis of normal cholangiocytes.
The extracellular proteinase Expi/Wfdc18 is induced by toll-like receptor 9 (TLR9) in macrophages and enhances hepatic fibrogenesis

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Introduction: We have identified quantitative trait loci (QTLs) that determine hepatocellular susceptibility to profibrogenic transforming growth factor (TGF)-beta signalling in cultured primary hepatocytes from the murine BXD reference population (Liebe et al. Physiological Genomics 2013). The Expi/Wfdc18 gene was identified as a potential candidate by eQTL analyses and the presence of a functional DNA variant. A knockout mouse was provided by the EUCOMM repository and showed decreased collagen contents following 6 weeks of CCl4 challenge.

Methods: To elucidate how Expi/Wfdc18 mediates the observed effect on hepatic fibrogenesis, we performed database and literature analyses for Expi/Wfdc18 regulation and function.

Results: A microarray experiment describing global changes in gene expression and synergistic interactions via TLR9 and TLR3 showed an impact of TLR9 on Expi/Wfdc18 expression: The transcript is significantly upregulated in RAW 264.7 macrophages following treatment with unmethylated CpG oligonucleotide, a TLR9 ligand, but not after treatment with the TLR3 ligand poly I:C. A synergistic induction by the combination of CpG oligonucleotide and poly I:C was observed (Tross et al. J Immunol. 2009). The immediately adjacent, highly homologous (70% amino acid identity) Wfdc17/AMWAP gene product is a negative regulator of macrophage activity in microglia and shows antibacterial activities (Karlstetter et al. J Immunol. 2010), suggesting a related function for Expi/Wfdc18 in the liver. Expression of TLR9 is elevated in cholangiocytes of patients with PBC and chronic HCV infection, while TLR3 and TLR4 are reduced (Benias et al. Clin Res Hepatol Gastroenterol. 2012).

Discussion/Conclusion: Expi/Wfdc18 is involved in hepatic fibrogenesis following chronic CCl4 challenge in the mouse. The expression of Expi is induced by unmethylated CpG via TLR9 and synergistically increased by poly I:C via TLR3 in a murine macrophage cell line. We conclude that Expi/Wfdc18 is involved in the TLR9-mediated innate defence against invading microbial pathogens and primes liver cells for pro-apoptotic signals.
Lipid accumulation enhances the susceptibility for oxaliplatin-induced hepatic injury

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Introduction: Chemotherapy can affect non-tumor healthy hepatic tissues and cause histopathological and functional changes summarized as chemotherapy-associated steatohepatitis (CASH). Malignant tumors and the metabolic syndrome are frequent diseases worldwide. The majority of patients with obesity or type 2 diabetes develop hepatic steatosis, and in a subset of patients progressive inflammation leads to non-alcoholic steatohepatitis (NASH). Thus, in a significant number of cases chemotherapy is given to patients with fatty livers.

The aim of this study was to establish an in vitro model to study the cytotoxic effects of oxaliplatin on steatotic and control hepatocytes.

Methods and results: We applied an in vitro model of hepatic steatosis, which we have recently developed (Wobser et al. 2009). Namely, incubation of HepG2 hepatoma cells or primary human hepatocytes with non-toxic concentrations of free fatty acids led to a dose-dependant cellular lipid accumulation. Cells were also incubated with serial concentrations of oxaliplatin for 24 h in order to establish a non-toxic dose range (up to 0.5 µM) as assessed by microscopic images, LDH and transaminases leakage in the supernatants.

Next, we added a non-toxic concentration of oxaliplatin (0.5 µM) to control as well as lipid loaded cells for 24 h. Microscopic analysis and analysis of LDH, transaminases and mitochondrial activity (XTT assay) confirmed that oxaliplatin at this dose induced significant cytotoxicity and inflammation in steatotic cells. At higher concentrations oxaliplatin also induced toxicity in control hepatocytes, however, steatotic cells were even more vulnerable.

Conclusion: These data indicate that hepatocellular lipid accumulation increases the susceptibility for oxaliplatin-induced hepatic injury. Our novel in vitro model may be used to unravel the underlying mechanisms of this phenomenon as basis for therapeutic strategies to prevent CASH particularly in patients, who suffer from metabolic syndrome.
Early induction of CXC-, CC-chemokines and of cytokines before neutrophil granulocytes and macrophage recruitment in different regions of the rat liver after single-dose thioacetamide (TAA)-administration

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**Introduction**: Cholangiocarcinoma and inflammation seem to be intimately related. Single-dose thioacetamide (TAA)-administration induces inflammation and acute-liver-damage (ALD).

**Methods**: We studied sequence and recruitment of inflammatory-cells in different liver regions in relation to CXC- and CC-chemokines and cytokines expression in a model of ALD induced by administration of single-dose TAA to rats intraperitoneally. Furthermore, if inhibition of TNF-α could reduce organ damage (inflammation) through lowering inflammatory mediators (chemokines), was also investigated by treating human-monocyte-cell-line (U937) with TNF-α in the presence or absence of anti-TNF-α (infliximab [IFX]). Tissue sections were used for immunohistochemistry. RT-PCR and Western-blotting was performed for RNA and protein analysis, respectively.

**Results**: An early increase (3 h) in CXCL8/IL-8 levels was measured followed by a dramatic release in MCP-1/CCL2 (24 h) serum-levels after TAA-administration. Likewise, an early increase of specific-RNA of hepatic chemokines CXCL1/KC and CXCL8/IL-8 was found at 3 h, followed by an upregulation of CXCL5/LIX (6 h), CXCL2/MIP2 (12 h) and MCP1/CCL2 gene-expression at 24–48 h. Furthermore, an induction of pro-inflammatory-cytokines IFN-γ and IL-1β followed by IL-6 and TNF-α was observed with a maximum at 12 h.

By means of immunohistochemistry, an early (12–24 h) increase in number of only neutrophil-granulocytes (NGs) attached to and around portal-vessel-walls was observed, followed by increased numbers of mononuclear-phagocytes along the sinusoids.

Treatment of the U-937 cell-line with TNF-α increased the gene-expression of CXCL1/KC, CXCL8/IL-8 and CCL2/MCP1. Conversely, adding of anti-TNF-α (IFX) to the culture medium inhibited this up-regulation significantly.

**Discussion/Conclusion**: By summarizing, single-dose TAA-administration induces a sequence of events with a defined up-regulation of gene-expression of inflammatory chemokines and cytokines and a transient accumulation of NGs within the portal-area (around biliary-cells) and macrophages along the sinusoids throughout the liver. In addition, inhibition of these cytokines could reduce organ damage (inflammation) by lowering the cell-infiltration numbers. Periportal inflammation seems to precede cellular damage which could ultimately lead to cholangiocarcinoma.
FAT/CD36: The main responsible protein for fat accumulation in rat liver after direct single-dose irradiation?

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Introduction: Fatty liver disease is known to be prevalence in patients with cholangiocarcinoma. Irradiation-induced cell damage and fat accumulation is already known in other organs and poorly understood in liver. As the liver is the major metabolic organ, and irradiation is known to affect its metabolic pathways, this study aimed to investigate fat accumulation in rat liver and genes involved in transportation of fat into liver after selective liver irradiation (25 Gy) in comparison to sham-irradiated controls.

Methods: Hepatic lipid accumulation, mRNA, and protein were studied by Nile red staining, RT-PCR, and Western-blotting analysis, respectively.

Results: By means of Nile-Red staining, an increase of fat droplets was observed in irradiated rat liver after 12, 24 and 48 h. Likewise, TG and FFA level increased in irradiated rat liver in comparison to sham-irradiated controls. An early increase in the serum level of HDL-C, TG and cholesterol was measured after single dose irradiation followed by a decrease thereafter.

In parallel to hepatic TG content, the expression of fat metabolism-involved genes was changed at mRNA and protein levels after single dose liver irradiation. Hepatic mRNA expression of ACO-1, ACC-2, L-FABP and ApoC3 were decreased after irradiation compared to the sham-irradiated. The gene expression of FAT/CD36 (fatty acid translocase) was the highest among the studied genes which was also confirmed by Western blot.

A Bandshift assay showed binding of AP-1 and NF-κB to CD36 promoter, suggesting a contribution in regulating CD36 expression.

The results were further confirmed by 2D-gel electrophoresis of irradiated and sham-irradiated rat livers. The spots were detected in areas corresponding to the different isoforms of CD36.

Conclusion: In conclusion, irradiation induced periportal inflammation and fat accumulation in the liver showed cellular damage which could further lead to cholangiocarcinoma.
PRC2 inhibition may directly target putative cancer stem cells in biliary tract cancer cell lines

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Introduction: The polycomb repressive complex 2 (PRC2) has the ability to silence genes by trimethylation of histone 3 at lysine 27 (H3K27me3). Previous experiments gave evidence that H3K27me3-mediated gene silencing is important for acquisition and maintenance of a malignant stem cell-like status – inhibited by 3-deaza-neplanocin-A (DZNep, an S-adenosyl-L-homocysteine hydrolase inhibitor). The current project characterizes the effects of DZNep on biliary tract cancer (BTC) cell lines, particularly cancer stem cells (CSC).

Methods: Assays for cellular viability, proliferation and sphere formation were used to assess the cytotoxic effects of DZNep and its effects on anchorage-independent growth in eight BTC cell lines in vitro, respectively. Real-time RT-PCR was used to quantify mRNA levels of PRC2 components, PRC2 downstream targets and CSC markers.

Results: We confirmed the expression of the PRC2 core components ezh2, eed and suz12 in all cell lines by RT-PCR. Treatment with DZNep of these cell lines reduced the overall cell viability by about 20–45%. Anchorage-independent growth as a characteristic of stem cell-like subpopulation was lowered by treatment with DZNep by 80% in EGI-1 cells. Furthermore, treatment with 20 µM DZNep decreased the expression of ezh2, eed and suz12 by 60%. Similarly, the cyclines ccna2 and ccnb1 displayed a strong decline accompanied by reduced expression of the stem cell markers cd24, cd133 and epcam. Interestingly, we also found a 60% reduction of hotair expression; a long-noncoding-RNA involved in epigenetic regulation and over-expressed in colon CSC subpopulations. Preliminary data also suggest that DZNep treatment reduces the Aldefluor-bright population of putative CSCs by about 10%.

Discussion/Conclusion: Epigenetic modifications including H3K27 trimethylation by PRC2 are involved in growth of biliary tract cancer cells. PRC2 inhibition by DZNep seems to directly suppress the CSC subpopulation of BTC cells; thus, indicating potential approaches to selectively target these cells responsible for chemoresistance and recurrence.

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Changes in hepatic iron uptake in a rat model of thioacetamide-induced restorable hepatic damage

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Under physiological conditions, iron regulation is tightly controlled within the reticuloendothelial system. However, liver is known to be crucial for iron metabolism. The current study highlights the changes in hepatic iron uptake during liver damage and recovery. Thioacetamide (TAA) has been used extensively in the development of animal models of liver injury. Sprague Dawley rats were sacrificed 1, 3, 6, 12, 24, 48, 72 and 96 hours after an intraperitoneal injection of TAA. Blood, liver and spleen were removed, frozen in liquid nitrogen and stored at -80°C. Serum was used to measure circulating hepatic enzymes and serum iron levels. Hepatic cryostat sections were evaluated with immunohistochemistry and pearl staining. Total protein was used for Western blot analysis.

Liver damage was detected by increased levels of circulating hepatic enzymes; ALT, AST, AP, LDH and GLDH with a maximum increase at 24 and 48 hours followed by a decrease thereafter. Moreover, hematoxylin and eosin staining indicated an increased number of mononuclear cells infiltrating around portal fields after TAA administration. After a slight decrease (1–6 h), serum iron level increased dramatically parallel to liver damage (24–48 h) with a return to control level at 72 h to 96 h. In contrast, hepatic iron levels increased immediately (1 h) and remained elevated during the course of study. However, spleenic iron level tends to decrease early followed by a significant increase at 96 h. Hepatic but not spleenic stainable iron was detected by Pearl staining in rat liver 72 h after TAA-injection. Western blot analysis of total hepatic protein demonstrated an increase in iron uptake protein and iron storage protein expression after TAA-injection. An increased hepatic protein expression of the major iron export protein (ferroportin-1) was observed in parallel to the increased circulating iron levels.

Our results suggest that during acute liver damage iron is released by mature liver cells (mainly hepatocytes) which is taken up by liver cells in recovery phase. Serum iron may be transported back to liver to meet the energy requirements and/or stored in liver cells within FTL and FTH.
Isolation of human liver cells for the establishment of functional co-culture and tissue engineering liver models

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Introduction: Besides the primary human hepatocytes (PHH), the liver contains non-parenchymal cells (NPC) like Kupffer cells (KC), hepatic Stellate cells (HSC) and liver sinusoidal endothelial cells (LSEC). NPC play a central role in many pathophysiologies of the liver, such as drug induced liver diseases, inflammation and the development of liver fibrosis and cirrhosis. In recent years, NPC have become more relevant for the development of liver co-culture models and in tissue engineering. Therefore, the aim of the present study was the establishment of a protocol for the simultaneous isolation of NPC from human tissue in a high quality and purity.

Methods: Liver cells were isolated by a two-step EDTA/collagenase perfusion technique. Parenchymal and the NPC containing fraction were separated and purified by density gradient centrifugation. Using specific adherence properties as well as magnetic activated cell sorting (MACS) the NPC were separated. Subsequently the cells were identified and characterized by immunofluorescence stainings (IF).

Results: KC were obtained by their ability to adhere short term on cell culture plastics and identified by IF staining for CD68 and their affinity for the phagocytosis of latex beads. They were counted with 1.25 x 10⁶ cells/g liver and a purity of more than 80%. LSEC were separated using MACS beads for endothelial surface protein CD31. We received a CD31 positive cell fraction with an amount of 2.5 x 10⁵ LSEC/g liver of over 80% purity. CD31 negative cells were identified as HSC by GFAP, alpha SMA and Vimentin as well as their specific autofluorescence of retinol. HSC were isolated with a cell count of 3.4 x 10⁵ cells/g liver and a purity of more than 90%.

Discussion/Conclusion: We established a protocol for the simultaneous isolation of parenchymal and non-parenchymal human liver cells with a high purity. Further investigations for the establishment of culture conditions and the creation of functional co-culture models are in progress.
The study of polarimetric spectrophotometry as a possible screening tool for cholangiocarcinoma

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Introduction: Currently, there is no effective screening test for cholangiocarcinoma (CCC). Serum levels of carcinoembryonic antigen, CA19-9 are neither specific nor sensitive enough for this purpose. However, it is well known that physical and chemical features of blood under CCC undergo significant changes. The aim of the study was to clarify the possibility of serum polarimetric spectrophotometry use for CCC screening purpose.

Methods: 74 patients participated in the study. Among them, 29 with CCC risk factors (ASH, liver cirrhosis, opisthorchiasis, hepatitis B or hepatitis C) – group 1. Results obtained in three CCC patients were used as exemplar – group 2. Patients with acute cholecystitis formed comparison group 3. Modified by polarization filter (90, 180, 270 degrees) serum spectrophotometry in ultraviolet (200–400 nm), visible (400–760 nm) and infrared (> 760 nm) spectra was performed.

Results: No significant difference in ultraviolet and visible spectra between groups was observed except 90 and 270 degrees polarization, while acute cholecystitis and CCC samples showed 18.92 ± 2.36% and 26.38 ± 2.11% higher values than group 1 (p < 0.05). Infrared spectrum (830 nm) absorption rate in CCC group was 1.74–3.05 times higher in all polarization planes compared to other groups, whilst no significant difference between groups 1 and 3 was found. Prevalence (0.067568), sensitivity (0.6) and specificity (0.934291) were comparatively high for values 15% over comparison group.

Discussion/Conclusion: High molecular weight molecules usually determine changes in infrared spectrum. Changes of their secondary and tertiary molecular structure may cause switches of polarization plane. While prevalence, sensitivity and specificity of the test were high, this study has important limitation caused by small number of tested objects.
Growth of human cholangiocellular carcinoma cells and expression of proangiogenic factors is affected by Nexavar treatment

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Introduction: Cholangiocellular carcinoma (CCC) is a rare disease, but its worldwide incidence and mortality rates are rising. CCC arises from intra- and extrahepatic bile ducts. Most patients are diagnosed at an advanced stage of disease resulting in poor prognosis and limited therapeutic options. To overcome this hurdle, we investigated whether treatment with Nexavar, a multiple tyrosine kinase inhibitor, could control CCC cell growth in vitro via the same mechanisms known for HCC treatment.

Materials and methods: Proangiogenic expression profiles (VEGFA/B/C/D, VEGFR1/2, Nrp1/2, EGF, EGFR) of two CCC cell lines (EGI-1 and MZA-Ch2) were determined by semiquantitative real time PCR. The cells were treated with 0, 1, 2.5, 5 and 10 µM Nexavar and proliferation, apoptosis, phosphorylation of signal transduction molecules (ERK1/2, p38 MAPK) and expression of the proangiogenic factors were analysed.

Results: Profiling of the CCC cell lines showed that both expressed extensive amounts of VEGF, EGF and EGFR. Treatment of CCC cells with Nexavar showed decreased proliferation and increased apoptosis in a dose dependent manner. Furthermore, phosphorylation of ERK1/2 and p38 MAPK was reduced in Nexavar treated EGI-1 and MZA-Ch2 compared to the DMSO-control as assessed by western blot. Analysis of the SubG1-fraction showed increased apoptosis following Nexavar treatment compared to DMSO alone in both cell lines. Expression of proangiogenic factors (VEGFA/B/C/D, VEGFR1 and Nrp1/2) was increased after Nexavar treatment in EGI-1 cells, whereas EGFR expression was decreased. For MZA-Ch-2 cells, Nexavar reduced the expression of the proangiogenic factors VEGFA/B/C/D, VEGFR1, Nrp1/2 and EGFR.

Conclusion: Here, we show that treatment with Nexavar reduces proliferation of cholangiocellular carcinoma cells and induces apoptosis in vitro by affecting intracellular signal transduction. Furthermore, Nexavar influences the expression of proangiogenic factors in both cell lines. Treatment with e.g. siRNA targeting proangiogenic factors could increase the antitumoural effects.
Changes in serum protein profile due to iron- and copper-induced toxicity in *Rattus norvegicus*

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**Introduction**: Iron (Fe) and copper (Cu) contribute in a broad range of usual metabolic processes and their requisite levels in the body are crucial for sustaining the normal health. Both elements are essential for the variety of enzymes to execute their functions properly and some proteins have direct effect on both Fe & Cu metabolism. However, as the levels of Fe & Cu increase beyond the normal range, the excessive amounts induce toxicity, directing to the harmful effects in the body. The aim of the current study was to compare the serum protein profile of treated and control groups by performing the Sodium-Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE) and mainly focused on acute phase proteins (APPs) for analysis.

**Materials and methods**: The experiment was conducted on male Wistar Rats (200 g) and three experimental groups were established against a control group. Group-I received 60 mg/kg FeSO₄, group-II received 10 mg/kg CuSO₄, and group-III received combined doses of Fe and Cu i.e., 30 mg/kg FeSO₄ & 5 mg/kg CuSO₄ while control group received only distilled water. Acute conditions were induced via intraperitoneal route and animals were sacrificed 24 h after Fe & Cu administration. Blood samples were collected and processed to isolate serum. Then serum proteins with different molecular weights were separated on SDS-PAGE. The stained gel was photographed, followed by the densitometric analysis using TotalLab™ Quant v12.3.

**Results**: Densitometric analysis of electrophoretically resolved low and high molecular weight serum protein fractions revealed different trends in experimental groups as compared to the control. The protein fractions of Transthyretin (15.78 kDa), Retinol binding protein (23.22 kDa), Albumin (66 kDa) and Transferrin (76.37 kDa) exhibited the decreasing trend in the experimental groups; whereas the Ceruloplasmin (122.22 kDa) protein fraction was observed to be markedly elevated in the experimental groups, when compared with the control group.

**Conclusion**: From the above findings, it is inferred that acute conditions of Fe & Cu overload elicited the toxic effects and caused alterations in serum protein profiles, verifying the inflammatory conditions. Acute phase response (APR) was confirmed by analyzing the considerable changes in concentrations of APPs in serum.
Histopathological and histochemical alterations due to acute iron and copper intoxication in rat liver

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Introduction: Iron (Fe) and copper (Cu) are essential trace elements; indispensable for a variety of metabolic processes and are needed at low levels to maintain normal health. Oxidative stress and free-radical reactions are the possible outcomes of the Fe & Cu excess and free-radical mediated cellular damage can be characterized by altered structure and function of the cells. As liver is the central organ associated with system Fe & Cu homeostasis; hence hepatotoxicity could be anticipated due to their acute exposure. The present study was aimed to investigate the changes in histopathology and histochemistry in live tissues of rat as a result of acute Fe & Cu intoxication.

Methods: The experiment was conducted on adult male Wistar Rats (200 g) and three experimental groups (n = 3) were established against a control group. Group-I received 60 mg/kg FeSO\(_4\), group-II received 10 mg/kg CuSO\(_4\), and group-III received combined doses of Fe & Cu i.e., 30 mg/kg FeSO\(_4\) & 5 mg/kg CuSO\(_4\) while control group received only distilled water. Animals were sacrificed after 24 h of acute intra-peritoneal administration of Fe and Cu. Liver was excised, cut and fixed in 10% formalin solution. Fixed tissue samples were dehydrated with graded ethanol, cleared with xylene, embedded in paraffin and finally sectioned and stained with hematoxylin and eosin for histopathological analysis. Prussian blue iron and Rhodanine stainings were done for histochemical analysis.

Results: Histopathological examination revealed severe signs of inflammation in the hepatic tissues of the treated groups that include infiltration of inflammatory cells at the hepatic portal regions. Variations in hepatocytes included cytoplasmic eosinophilia, necrosis, binucleation, hydropic degeneration and cytoplasmic ballooning. Moreover Kupffer cells hyperplasia and dilation of portal veins and sinusoids were demonstrated. Histochemical evaluation revealed massive hepatic iron accumulation in the iron treated groups, whereas hepatic copper deposition was not demonstrated in the experimental groups.

Conclusion: Taken together these findings it is concluded that acute exposure to Fe and Cu caused significant histopathological and histochemical variations, thus specifying the inflammatory conditions, directed to hepatotoxicity.
Impact of acute iron & copper intoxication on biochemical liver function in Wistar rat

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Introduction: Various indispensable metabolic processes are reliant on minute amounts of essential trace elements i.e., iron (Fe) and copper (Cu) and on Fe-Cu association as well to maintain normal health. As liver holds central importance in both Fe & Cu homeostasis, therefore their exceeding quantities can render abnormality in liver function. The aim of this work was to evaluate the acute hepatotoxicity of Fe & Cu by analyzing the biochemical functions of liver.

Materials and methods: Adult male Wistar rats (200 g) were used and three experimental groups (n = 3) were established against a control group i.e., G-I: 60 mg/kg FeSO$_4$; G-II: 10 mg/kg CuSO$_4$; & G-III: 30 mg/kg FeSO$_4$ & 5 mg/kg CuSO$_4$ while control group (Con) received only distilled water. Doses were administered via intra-peritoneal route and animals were sacrificed after 24 h. Blood samples were collected and processed to isolate serum. Serum samples were analyzed for liver function tests (ALT, AST, ALP, albumin, total protein and bilirubin total) through using DiaSys Kits as per manufacturer’s instructions.

Results: Experimental groups showed statistically significant elevation in ALT activity (p < 0.0001), AST activity (p = 0.0012), ALP activity (p = 0.0001), bilirubin total concentration (p = 0.0245) and a significant decline in albumin (p = 0.0011) and total protein concentration (p = 0.0002), as compared to the control, when analyzed by one-way ANOVA. The results are illustrated in table 1.

Table 1: Serum biochemistry: Results indicate mean value ± S.E.M. (Level of Significance p < 0.05*; 0.01**; 0.001*** analyzed by one-way ANOVA with post-hoc Tukey test).

<table>
<thead>
<tr>
<th>Groups</th>
<th>ALT (U/l) $\bar{x}$ ± s.e.m</th>
<th>AST (U/l) $\bar{x}$ ± s.e.m</th>
<th>ALP (U/l) $\bar{x}$ ± s.e.m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>37.67 ± 1.20</td>
<td>38.00 ± 3.21</td>
<td>54.00 ± 8.50</td>
</tr>
<tr>
<td>G-I</td>
<td>28.67 ± 1.66</td>
<td>29.67 ± 4.09</td>
<td>168.0 ± 3.00 a*</td>
</tr>
<tr>
<td>G-II</td>
<td>185.0 ± 17.0 a***, b***</td>
<td>146.3 ± 21.65 a**, b**</td>
<td>337.3 ± 36.59 a***, b**</td>
</tr>
<tr>
<td>G-III</td>
<td>76.50 ± 11.5 a*, b*, c***</td>
<td>144.3 ± 24.54 a**, b**</td>
<td>291.0 ± 10.69 a***, b*</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Groups</th>
<th>Albumin (g/dl) $\bar{x}$ ± s.e.m</th>
<th>Total Protein (g/dl) $\bar{x}$ ± s.e.m</th>
<th>Bilirubin Total (mg/dl) $\bar{x}$ ± s.e.m</th>
</tr>
</thead>
<tbody>
<tr>
<td>Con</td>
<td>6.20 ± 0.10</td>
<td>10.25 ± 0.45</td>
<td>0.30 ± 0.57</td>
</tr>
<tr>
<td>G-I</td>
<td>4.50 ± 0.30</td>
<td>7.95 ± 0.05 a**</td>
<td>0.56 ± 0.03</td>
</tr>
<tr>
<td>G-II</td>
<td>3.30 ± 0.10 a***, b*</td>
<td>6.66 ± 0.12 a***, b*</td>
<td>0.50 ± 0.10</td>
</tr>
<tr>
<td>G-III</td>
<td>4.15 ± 0.05 a**</td>
<td>6.70 ± 0.26 a***, b*</td>
<td>0.63 ± 0.06 a*</td>
</tr>
</tbody>
</table>

a: significance level when compared to control. b: significance level when compared to Group I. c: significance level when compared to Group II.
**Conclusion:** It is deduced from the above investigations that acute exposure to Fe & Cu and their co-administration caused noticeable alterations in liver biochemical functions, thereby signifying as indicators for the severe hepatotoxicity during acute Fe & Cu overload conditions.
Genetically engineered Upcyte® hepatocytes as a capable application for energy metabolism experiments in *in vitro* liver cell systems

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**Introduction:** The use of primary human hepatocytes (PHH) in biomedical research is still considered as the gold standard. Beside hepatic cell lines and stem cell derived hepatocyte like cells, genetically engineered cells have become a new promising approach for methodical supplementation in biomedical research. Aim of the present study was the characterization of a genetically engineered hepatocyte culture in comparison to conventional liver model systems.

**Methods:** PHH were isolated from human liver resectates using a two-step collagenase perfusion technique. A lentiviral vector system was used to transfer proliferation genes (Upcyte® genes) to generate a stable hepatocyte culture (HepaFH3). PHH, HepG2 and HepaFH3 were analyzed for their capability of lipid and glucose storage using Oil-Red-O staining and a biochemical assay for glycogen quantification, respectively. Energy consumption was investigated by measurement of mitochondrial activity using the MTT assay. For evaluation of hepatic functions: albumin synthesis, urea formation and transaminase activities were determined.

**Results:** Energy consumption of PHH showed lowest rates whereas two fold higher rates in HepaFH3 and up to ten times higher rates in HepG2 were detected. PHH showed gluconeogenesis, while HepG2 cell medium was completely depleted of glucose after 24 h. Glycogen storage revealed a fourfold lower rate in differentiated HepaFH3 than in PHH, a 100 fold lower value in proliferating HepaFH3 and was not detectable in HepG2 cells. Investigation of hepatic functions displayed a diminished capability of cell lines to synthesize urea and albumin, as well as diminished transaminases activities. However, in comparison with cell line HepG2, HepaFH3 loses capability of albumin and urea synthesis with increasing number of cell passages.

**Discussion/Conclusion:** HepaFH3 is showing more consistent values in glucose metabolism with PHH compared to HepG2, therefore HepaFH3 seem to be a more appropriate substitution for PHH in *in vitro* experiments concerning energy metabolism.
Inhibition of cyclooxygenase-2 in vitro induces apoptosis and decreases proliferation of the human cholangiocarcinoma cells

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Introduction: Several studies showed that selective cyclooxygenase-2 (COX-2) inhibitors suppress growth of several cancer cells and have chemopreventive potential during colon and liver cancerogenesis. However, it is still unclear whether COX-2 contributes to the malignant growth and how inhibition of COX-2 modifies the malignant potential of cholangiocarcinomas. The aim of the study was to clarify the pro-apoptotic and anti-proliferative effect of selective COX-2 inhibition of human cholangiocarcinoma cells.

Methods: CCC cell line HuCCT-1 was cultivated in modified medium with 10% fetal bovine serum seeded onto well plates. Celecoxib 50 μmol/l added in study group cultures. Apoptosis related cytokines were analyzed by Western blotting. Apoptotic nuclei were visualized with the TUNEL-staining protocol and cells viewed with a fluorescence microscope (magn. ×400). The number of apoptotic cells calculated in percentage of total nuclei.

Results: COX-2 inhibition related changes become evident in HuCCT-1 cell lines after 48 hours of treatment leading to a significant time-dependent reduction of cell numbers of up to 75% (p < 0.05). Cells became sparse, rounded, and detached from the dishes representing morphologic signs of apoptosis. This correlated with activation of caspase-9, caspase-3, and caspase-6 cytokines. However, exposure of cell cultures to 3 g/ml PgE2 eliminated the COX-2 inhibiting and pro-apoptotic effect on cells. This indicates that the antineoplastic properties of COX-2 inhibiting are dependent on reduced conversion of arachidonic acid to PGE2 attributable to COX-2 inhibition.

Discussion/Conclusion: Selective inhibition of COX-2 causes marked growth inhibition of human CCC, based on the induction of apoptosis and inhibition of proliferation. The mechanism by which COX-2 inhibiting-related apoptosis is realized is still unclear as well as involvement of other factors into antiproliferative effect of COX-2 inhibitors. The data obtained in this study support our previous findings of COX-2 inhibitors role in cancer cells proliferation suppressing.
In vitro study of vascular endothelial growth factor inhibition in cholangiocarcinoma

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Introduction: Inhibition of vascular endothelial growth factor (VEGF) signaling may affect tumor growth of different origin through several mechanisms. Studies involving cholangiocarcinoma (CCC) are extremely rare and present various and often disappointing results. We hypothesized that VEGF inhibiting may be similarly effective on CCC as on hepatocellular carcinoma.

Methods: CCC cell line HuCCT-1 was cultivated in modified medium with 10% fetal bovine serum seeded onto well plates. VEGF-targeting drug Sorafenib 0.05 mg/ml added in study group cultures. General cells count and nuclei morphology were visualized with the TUNEL-staining protocol and cells viewed with a fluorescence microscope (magn. ×400). The number of apoptotic cells calculated in percentage of total nuclei. Apoptosis related cytokines were analyzed by Western blotting.

Results: Changes after addition of Sorafenib become evident in HuCCT-1 cell line compared to control after 48 hours of treatment leading to a significant time-dependent reduction of cell numbers of 59.6–82.4% (p < 0.01). Cells became sparse, rounded, and detached from the dishes representing morphologic signs of apoptosis. This correlated with activation of caspase-9, caspase-3, and caspase-6. These changes were similar to changes observed in hepatocellular carcinoma Hep G2/Hep 3B lines used for control with comparable pro-apoptotic effect during same time interval.

Discussion/Conclusion: VEGF-targeted therapy may act through parallel mechanisms that have more or less important role depending on tumor type. In certain malignancies VEGF-targeted therapy has significant activity, whereas in other has no clinical benefit. Our study shows positive pro-apoptotic effect of VEGF-targeting therapy. However, there is no evidence that other CCC cells lines will be similarly sensitive for VEGF-targeting therapy as HuCCT-1.
In vitro influence of peroxisome proliferator-activated receptor-gamma activation on cholangiocarcinoma cells

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Introduction: Peroxisome proliferator-activated receptor-gamma – NR1C3 (PPARG) plays an important role in various biological processes including lipid and glucose metabolism. PPARG agonists have been used in treatment of different metabolic disorders and Nonalcoholic Steatohepatitis decreasing steatosis, inflammation, and fibrosis. Recent studies show its pro-apoptotic and antiproliferative effect. The aim of the study was to clarify the perspectives for cholangiocarcinoma (CCC) targeted therapy with thiazolidinediones.

Methods: CCC cell line HuCCT-1 was cultivated in modified medium with 10% fetal bovine serum seeded onto well plates. PPARG agonist pioglitazone 0.5 to 10 mmol/L added in study group cultures. General cells count and nuclei morphology were visualized with the TUNEL-staining protocol and cells viewed with a fluorescence microscope (magn. ×400). The number of apoptotic cells calculated in percentage of total nuclei. Apoptosis related cytokines were analyzed by Western blotting.

Results: Activation of PPARG by pioglitazone caused marked growth inhibition in a time- and dose-dependent manner. Pioglitazone inhibited growth of cholangiocarcinoma cell lines by inducing apoptosis and by cell cycle regulation, and this was associated with caspase-3, 6 and caspase-9 activation. These changes were similar to changes observed in hepatocellular carcinoma Hep G2/Hep 3B lines used for control with comparable pro-apoptotic effect during same time interval but different pioglitazone doses.

Discussion/Conclusion: PPARG activation shows positive pro-apoptotic and antiproliferative effect on CCC as well as other tumour types. Molecular targeting with thiazolidinediones, nuclear receptor ligands, may be a promising strategy for treating cholangiocarcinoma. However, our previous study of PPARG agonists and hepatocellular carcinoma raised question of individual susceptibility/resistance for this approach.
**MicroRNAs associated with the efficacy of photodynamic therapy in biliary tract cancer cell lines**

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**Introduction**: Photodynamic therapy (PDT) is established for palliative treatment of non-resectable hBTC (hilar biliary tract cancer) with a considerable benefit for survival and quality of life. The specific factors determining the cellular response of biliary tract cancer cells towards PDT are still unknown. Due to their multifaceted functions, microRNAs (miRs) are a promising analyte to investigate the mechanisms governing sensitivity and resistance.

**Methods**: For two photosensitizers, Photofrin® and Foscan®, the cellular sensitivity towards in vitro PDT was investigated in eight BTC cell lines using the resazurin viability test. Each cell line (untreated) was profiled for expression of n = 754 miRs using quantitative RT-PCR (TaqMan® Array Human MicroRNA A+B Cards v3.0, Applied Biosystem). Statistical analysis was used to identify miRs associated with PDT efficiency. Furthermore, correlation analysis of miR expression and markers of proliferation and differentiation (Ck19, Ck8/18, vimentin, cyclin D1 and E-cadherin) was performed.

**Results**: We identified eleven miRs significantly positively correlated with the viability post PDT ('resistance miRs') and eight miRs with negative correlation ('phototox miRs'). PDT was particularly effective in cells with high levels of clustered miRs 25-106b and in case of miR-106b a phenotype characterized by high expression of the mesenchymal marker vimentin and high proliferation (cyclinD1 and Ki67 expression). A PDT-resistant phenotype displays high expression of miR-200 family members and in case of miR-cluster 200a/b-429 expression of differentiation markers Ck19 and Ck8/18. Bioinformatic analysis of predicted and validated downstream targets suggests direct involvement of miR-200c and -203 in response mechanisms to oxidative stress, nitric oxide biosynthesis and in stress-activated signaling.

**Discussion/Conclusion**: The heterogeneity of the PDT response is associated with a distinct miRNome pattern providing a novel tool for predicting the efficiency of PDT. Following confirmation by subsequent mechanistic approaches, this may allow optimization of PDT protocols to develop more reliable and efficient treatment regimens.

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No liver malignancies in the HCV-1b (anti-D) Leipzig cohort after more than three decades

Manfred Wiese and Franziska Richter
East German HCV Study Group, Leipzig, Germany

Introduction: The natural course of the hepatitis C virus genotype 1b (HCV-1b) infection is still unclear but important for the decision whether an interferon-based therapy with the possibility of severe adverse events should be initiated. It was assumed that 30% of patients have been developed a liver cirrhosis with subsequent liver malignancies after 20 years. Up to now there are very few unbiased long-term follow-up studies with known dates of infection.

Methods: 14 HCV-1b contaminated batches of anti-D immunoglobulin had been administered to 2867 women throughout East Germany in 1978/79. In an earlier report we published 0.5% cirrhosis after 25 years. 181 women of the Leipzig cohort could be followed after 30 years. We show the frequency of severe liver damages, malignancies and end-points (death, LTx) in 133 women (48 excluded because of antiviral therapy) after more than 30 years.

Results: After more than 30 years, 33% of the 354 affected women still tested positive for HCV RNA. Only eleven of the HCV positive pts. (8.3%) developed a liver cirrhosis, 7 women (5.3%) precirrhotic stages. In the last 15 years, a continuous, but low increase of fibrotic scores was observed. Up to now no liver malignancy was diagnosed. Six women of the Leipzig cohort died of HCV related complications while seven pts. (HCV eliminated) died of other diseases like cardiovascular diseases or accidents. Between the 2 groups, there was no statistical difference.

Discussion/Conclusion: Young women without comorbidity may clear HCV (1b) infection in more than half of the cases, or develop mild chronic hepatitis C. We confirm the low risk of progression to cirrhosis and to subsequent liver malignancies in the Leipzig HCV-1b cohort within more than three decades.
Decline of DNA repair ability is paralleled by decreased autophagy in rat aging liver

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Introduction: The AMPK-PTEN-mTOR pathway is related to autophagy and thus plays a role in the aging process. Furthermore, AMPK and p53 are closely related to the ability of DNA repair, of which OGG-1, XRCC-1, and PARP-1 are important mediators. However, it is unknown, whether a cross-talk between DNA repair components and autophagy exists in the aging liver.

Methods: Nine Wistar rats divided into 3 groups were used: young (6–8 weeks), middle-aged (6–8 months) and old (23 months). The molecular mechanism of cross-talk between DNA repair and autophagy in rat liver tissue was demonstrated by Western blot.

Results: The protein expression pattern of Atg 5, 12 and 16 was significantly lower in old rat liver tissue. It was accompanied by a significant decrease of DNA repair components (XRCC-1, OGG-1, and PARP-1). P53, AMPK, and the PTEN-Akt-mTOR pathway showed different expression patterns in livers of different age: Expression of p53 was significantly higher in the livers of old rats than in livers of young and middle-aged rats. Furthermore, AMPK expression in middle-aged and old rats was significantly lower than in those of the young group. In contrast, the downstream expression of PTEN in middle-aged and old rats was significantly higher than in younger ones.

Discussion: In the present study, our data have illustrated that an over-expression of p53 is able to affect the AMPK and PTEN in liver tissue, which indirectly affects mTOR pathway. Low expression of mTOR reduces the formation of autophagosome in rat liver tissue. In old rat livers also showed a reduced expression of DNA repair accompanied by a decreased Atg expression. This phenomenon indicates that DNA repair components gradually reduce their repairing ability on the gene sequence of Atg (deletion and mismatch) with increasing liver age.

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