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SELECTION OF THE MONTH

Big Title: Non-selective beta blockers IMPROVE survival of ACLF patients

Small Titles:

Beyond hNTCP – the missing link to support fully permissive immuno-competent HBV mouse models

New Practice guidelines for NAFLD

A nanoparticle-based therapy to decrease portal pressure

ALCOHOLIC LIVER DISEASE (ALD)

Hepatocytes exposed to alcohol drive inflammation, the proto-oncogene c-myc promotes disease initiation and progression

The mechanisms by which hepatocyte exposure to alcohol results in stimulation of resident macrophages (i.e., Kupffer cells) and/or recruitment of monocytes are

unknown. Verma *et al.* were interested in CD40 ligand (CD40L, also known as tumor necrosis factor (TNF) ligand superfamily member 5) whose interaction with CD40 (known as TNF receptor superfamily member 5) triggers the inflammatory NF-kappaB pathway. They show that when exposed to alcohol, hepatocytes overexpressing alcohol-metabolizing enzymes release, in a caspase-dependent manner, extracellular vesicles containing CD40L, which can stimulate macrophages to produce inflammatory cytokines. **These results suggest a new model in which hepatocyte injury/stress (the nature of which remaining elusive) promotes inflammation by stimulating resident macrophages.**

The proto-oncogene c-myc (encoded by *MYC*) is a multifunctional, nuclear phosphoprotein that plays a role in cell cycle progression, apoptosis and cellular transformation. It functions as a transcription factor that regulates transcription of specific target genes. The potential role of c-myc in the development of ALD is unknown. Here Nevzovora *et al.* address this question by using mice with transgenic expression of c-myc in hepatocytes that received or not alcohol. **They find that expression of c-myc and alcohol synergistically accelerates the progression of ALD presumably due to loss of p53-dependent protection.**

HEPATOCELLULAR CARCINOMA (HCC)

Hedgehog signaling, Laminin-332 and cancer stem cells, complete removal of tumor-bearing portal territory, blood transfusion (PBT) and HCC resection

The Hedgehog (Hh) family of secreted signaling proteins plays a crucial role in development of diverse animal phyla, from Drosophila to humans, regulating morphogenesis of a variety of tissues and organs. Hh signaling is also involved in control of stem cell proliferation in adult tissues and aberrant activation of the Hh pathway has been linked to multiple types of human cancer. Members of the Hh family bind to patched (ptc), thus releasing smoothened (smo) to transduce a signal. Transcriptional activation occurs through the GLI family of proteins resulting in activation of target genes. There are three human Hh proteins: Sonic hedgehog (Shh), Desert hedgehog (Dhh) and Indian hedgehog (Ihh). Little is known on the role (Hh signaling in liver fibrosis and HCC. Chung *et al.* using a transgenic model of Shh hepatic expression show that **this expression induces liver fibrosis with concurrent activation of hepatic stellate cells and fibrogenic genes. It can also enhance liver carcinogenesis induced by other oncogenes.**

Cancer stem cells (CSCs) may persist in tumors due to their chemoresistance and cause relapse and metastasis. Govare *et al.* using elegant approaches in HCC and under vivo and in vitro conditions, identify an important role for **laminin (Ln)-332 and more precisely its γ2-chain as part of the specialized CSC niche in maintaining and supporting 'stemness'.** The γ2-chain of Ln-332 could be a novel target in the treatment of HCC.

Although anatomic resection of the tumor-bearing portal territory has been reported to be associated with decreased recurrence of HCC, its oncologic advantage is controversial. Sindoh *et al.* address this question in a prospective series of 209 patients with Child-Pugh class A cirrhosis and solitary HCC of less than 5 cm. **They show that complete removal of tumor-bearing portal territory decreases risk of local recurrence and death from HCC.**

Several studies have reported on the negative effects of perioperative blood transfusion (PBT) on oncologic outcomes after curative resection of HCC. However, these findings are controversial. To address this issue, Yang *et al.* report the results of a retrospective cohort study including 1103 patients and investigated the relationship between PBT and long-term recurrence and survivals after curative resection of HCC using propensity score matching and multivariable Cox regression analyses. **They find no evidence for an influence of PBT on recurrence-free survival and overall survival after curative resection of HCC.**

NONALCOHOLIC FATTY LIVER DISEASE (NAFLD)

Molecular and genetic drivers of fibrosis in NASH, a nanoparticle-based approach is useful to decrease portal pressure

The development of liver fibrosis determines the progression to cirrhosis in patients with NASH. In this issue, Eguchi *et al.* studied the role of **Bid, a key pro-apoptotic molecule(encoded by *Bid*, for BH3 interacting domain death agonist)**, in experimentally-induced fatty liver in mice. By using different genetic approaches including hepatocyte-specific *Bid* deficient mice, the authors provide convincing evidence that Bid participates in fibrosis progression. These findings are consistent with existing evidence that **hepatocyte apoptosis triggers stellate cells activation** and liver fibrosis and suggest that Bid inhibition may be useful as an antifibrotic therapy in fatty liver diseases. In another article, Petta *et al.* were interested in *MERTK* (for MER Proto-oncogene, tyrosine Kinase; encoding the protein tyrosine

kinase Mer). Mer belongs to the family of TAM (for Tyro, Axl, Mer) receptors that are expressed at cell surface of macrophages and dendritic cells and play a role in apoptotic cell clearance (and thus tissue homeostasis) and inhibition of Toll-like receptor mediated inflammation. **Genetic variations in *MERTK*** which is known to regulate fibrosis in hepatitis C, are associated with the susceptibility of NASH patients to develop fibrosis. Petta *et al.* studied a large cohort of patients and experimental models of NASH. *MERTK* was overexpressed in the liver of NAFLD patients with F2-F4 fibrosis and in models of fibrogenesis and induce fibrogenic actions in stellate cells. Moreover, they demonstrated that the **rs4374383 AA genotype**, associated with lower intrahepatic expression of *MERTK*, **is protective against F2-F4 fibrosis in patients with NAFLD**.

In a third study, Oro *et al*. studied the beneficial effects of cerium oxide nanoparticles (CeO2NPs), a new pharmacological tool with potential antioxidant and anti-inflammatory properties. Systemic and hepatic effects were assessed in CCl_4-treated rats receiving nanoparticles or vehicle. Most CeO2NPs were located in the liver and **reduced hepatic steatosis, systemic and hepatic inflammatory biomarkers and portal pressure** without affecting mean arterial pressure. This novel approach may be of therapeutic value in advanced liver diseases and clinical trials are anticipated.

HEPATITIS B

Silencing hepatitis B virus (HBV) RNA, restoration of HBV specific T-cell response by combination therapy, beyond hNTCP – the missing link to support fully permissive immunocompetent HBV mouse models

The limitations of current licensed HBV oral drugs – the nucleos(t)ide analogs - are their rather limited effect on HBsAg production because HBV mRNAs derived from the nuclear cccDNA are continuously produced even under effective suppression of viral replication. Yamamoto *et al.* provide a novel and very sophisticated approach by developing short-interfering RNAs (siRNAs) targeting these HBV-specific RNAs in order to suppress production of HBV proteins. Hepatocyte specific delivery of the siRNA - one of the most challenging issues in silencing strategies - was realized by designing a novel pH-sensitive hepatocyte-specific multifunctional envelope-type nanodevice drug delivery system (MEND/HBV-siRNAmix). **MEND/HBV-siRNA caused a significantly more efficient reduction of HBsAg and HBeAg in primary human hepatocytes and in chimeric mice with humanized liver as compared to**

entecavir; and quite interestingly, the effect of a single nanodevice siRNA application lasted for two weeks making the MEND/HBV-siRNA an attractive and promising novel HBV antiviral candidate.

Multispecific HBV-specific T cell responses play an important role in acute, self-limiting HBV infection. T-cell exhaustion, in contrary, has been linked to the chronic stage, and is probably the result of repetitive T-cell receptor stimulation by persistently high HBV antigen levels. De Niet *et al.* performed extensive T-cell analyses in patients with partially controlled HBV infection (i.e. low-level HBV replication) but also patients with high HBV replication who have been treated with a combination of peginterferon plus adefovir. A relatively narrow HBV-core-specific T cell population with a resting effector-memory phenotype and strong proliferative potential, seem to be sufficient to retain the virus at low levels. Whereas **a partial recovery of HBV-specific T cells showing a broader repertoire was found in treatment induced HBsAg loss**, as well as in some patients on long-term nucleos(t)ide therapy. These data are important for a better understanding of the mechanisms behind T-cell dysfunction and treatment-induced restoration, and may also foster development of combination therapy strategies to enhance viral clearance.

The discovery of the hNTCP receptor as the main HBV entry receptor opened the avenue to establish new HBV replication models by rendering non-permissive cells permissive to HBV by the expression of hNTCP. The ability of heterokaryonic cells, generated by fusion of hNTCP-expressing mouse and human cell lines with replication-supporting but non-infectable HepG2, to fully support HBV replication was studied in a very elegant study by Lempp *FA et al.* The key finding were that first **despite hNTCP-expression the HBV replication was still restricted in mouse cell lines and the nonhepatic human HeLa cells**, but second these NTCP-independent restrictions of HBV infection can be overcome by cell fusion mediated complementation with an intracellular factor expressed in HepG2 cells. Identification of this missing link will be a major milestone on the way towards the development of an immunocompetent mouse model for HBV infections.

HEPATITIS E

New stem-cell derived hepatitis E virus (HEV) replication model

Relatively little is known about the molecular biology of HEV infection and replication as i*n vitro* HEV culture systems have only recently been established. The mostly transformed cell lines that are used so far, like hepatoma cell lines Huh7, HepG2, HepaRG, and others are, however, physiologically less relevant to study HEV replication than primary hepatocytes, which are however not readily available. Helsen *et al.* **reported now for the first time, the infection of human pluripotent stem cell-derived hepatocytes by HEV supporting the complete viral replication cycle**, and propose this model as an alternative and physiological cell culture model for the study of HEV infection and inhibition thereof by antiviral drugs. The new stem cell-derived HEV replication model represents an important tool for the future investigation of HEV cell tropism and designing novel therapeutic strategies.

CIRRHOSIS

Non-selective beta-blockers (NSBBs) improve the survival of ACLF patients

NSBB's have been the mainstay of pharmacological therapy for portal hypertension in cirrhotic patients for over 25 years. Data in the past few years have indicated that mortality of patients with refractory ascites or spontaneous bacterial peritonitis may be increased if NSBBs are continued. The important paper by Mookerjee *et al.* analyses the data obtained in the prospective observational study in patients with acute deterioration of cirrhosis requiring hospital admission, the CANONIC study. The results show that the **patients that were being treated with NSBBs at the time of hospital admission with ACLF had a significantly lower mortality, which was associated with less severe inflammatory response.** However, the doses of NSBBs used was relatively low suggesting that the protective effect may be due to mechanisms other than its known hemodynamic effects. The data argue against stopping NSBBs in cirrhotic patients unless there are specific contraindications.

PRIMARY SCLEROSING CHOLANGITIS

Inhibition of bile acid absorption reduces severity of bile duct injury in a mouse model

In the $MDR^{-/-}$ mouse, features of sclerosing cholangitis are known to be associated with altered bile acid composition, which after biliary excretion undergoes enterohepatic circulation and over 95% is reabsorbed in gut. As these toxic bile acid can result in further bile duct injury, reduction in their reabsorption can prevent further

bile duct injury. Trauner *et al.* hypothesized that blocking the ASBT/SLC10A2 receptor, which is known to take up about 95% BA's from the gut would prevent bile duct injury. **The authors used A4250, a specific ASBT inhibitor and showed clearly that its use was associated with significant attenuation in the severity of bile duct injury providing exciting new data that can potentially be translated into clinical practice.**