Background and Aims: Intestinal bacteria frequently possess flagella, whose structural protein, flagellin, is sensed by the human toll-like receptor 5 (TLR5). Bacterial translocation from the gut is an important mechanism of liver disease progression by stimulating immune response. We wondered whether a variant in a functional TLR5 polymorphism affects development of hepatocellular carcinoma (HCC).

Method: Healthy controls, patients with alcohol abuse but without significant liver disease, and three cohorts of patients with cirrhosis due to alcohol-related or non-alcoholic steatohepatitis with and without HCC were genotyped for the non-synonymous rs5744174 polymorphism in the TLR5 gene. Levels of inflammatory cytokines of stimulated monocytes from healthy controls was enhanced in patients compared to healthy controls.

Results: Frequency of the TLR5 rs5744174 TT genotype was similar in healthy controls (n = 212; 33%), controls with alcohol abuse (n = 382; 34%), and patients with alcohol-related cirrhosis in the discovery cohort (n = 372; 28%), the validation cohort (n = 355; 33%) and the cirrhosis cohort of non-alcoholic steatohepatitis (NASH) (n = 139; 28%). However, when patients were stratified according to presence of HCC (n = 79; n = 132; and n = 61 in the respective cohorts), prevalence of the TT genotype was significantly higher in cirrhotic patients with compared to without HCC in the discovery (41% vs 25%), the validation (39% vs 29%) and the NASH cohort (39% vs 22%) (each p < 0.05). The association between presence of the TT genotype and HCC remained significant (OR = 1.8; CI 1.1–2.7; p = 0.01) after multivariate correction for age (OR = 1.09/year; CI 1.06–1.12; p < 0.0001), gender (OR = 3.4; CI 1.5–5.9; p < 0.0001), diabetes (OR = 2.2; CI 1.5–3.5; p < 0.001), and carriage of the PNPLA3 148M variant (OR = 2.2; CI 1.4–3.5; p = 0.001). Interleukin-8 response of flagellin-stimulated monocytes from healthy controls was enhanced in carriers of the TT genotype (p = 0.02). Patients with alcohol-related liver cirrhosis carrying the TT genotype had higher serum levels of interleukin-8 (mean 177 vs 61.8 pg/ml; p = 0.02) and of CXCL1 (mean 418.1 vs 220 pg/ml; p = 0.03).

Conclusion: The TT genotype of the rs5744174 polymorphism in the TLR5 gene is associated with an increased risk for HCC due to steatohepatitis, which may be linked to enhanced immune response to translocated flagellin.

Hepatitis B virus surface antigen inactivates the hippo pathway and thereby increases the hepatic expression of oncogenic BMI1

Xufeng Luo1, Mengji Lu2, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1, Bettina Langhans1, Felix Sticke1, Jacob Nattermann1

Method: Reanalysis of GEO (GSE84429) data was performed to search for eligible pathways associated with HBsAg overexpression. PMH were isolated from HBsAg-transgenic or wildtype mice and analyzed by flow cytometry and western blot. Immunocytochemistry staining was performed to intracellularly locate YAP and BMI1 proteins. Dual-luciferase reporter (DLR) assay, chromatin immunoprecipitation (ChIP) was performed to prove the direct regulation of the Bmi1 promoter by the YAP/TEAD4 transcription factor complex. Short hairpin RNA (shRNA)-induced gene knockdown and overexpression of selected factors were investigated.

Results: Reanalysis of Chip data showed that genes associated with Hippo signalling, cell cycle, centrosomal function and DNA repair/replication were altered in HBsAg-transgenic mice liver. Quantitative PCR and western blot results confirmed that downregulation of MST1/2 is accompanied by loss of YAP phosphorylation and induction of BMI1 expression. Immunohistochemical staining of HBsAg-transgenic mice liver further indicated that expression of HBsAg induced an increase of YAP and BMI1, which resulted in cell proliferation and the abnormal nucleus morphology. Flow cytometry revealed that ploidy and aneuploidy also occurred in PMH of HBsAg-tg mice. Bioinformatic analysis of Bmi1 promoter indicated the presence of several TEAD4 bind sites. ChiP assay and binding site mutated DLR assays confirmed that YAP/TEAD4 transcription factor complex bound and activated the Bmi1 promoter. Hepa1-6 cell line was transfected with an RFP-reporter plasmid containing the Bmi1 promoter region, herein knockdown of YAP or Tead4 led to decreased RFP signals.

Conclusion: The Hippo signalling pathway plays a vital role in cell homeostasis. Our findings led to suggest that HBsAg-mediated Hippo pathway inactivation results in BMI1 expression, possibly promoting hepatocarcinogenesis through alteration in cell cycle and chromosomal stability.

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and siRNA knockdown of XPO1 in HUH7 cells reduced activation of KIR2DS2. Vaccination of KIR-transgenic mice with a KIR2DS2-targeting DNA vaccine increased activation of NK cells in both spleens and livers as determined by KLRE1 expression (p < 0.01), and this was most marked on mature CD11b+CD27-KIR2DS2+ NK cells (p < 0.01). NK cells from vaccinated mice had peptide-specific NK cell responses in vitro, which were not observed in peptide control vaccinated mice. Adoptive transfer of NK cells from vaccinated mice led to impaired growth of HUH7 cells in NOD/SCID/γ, KO mice, as compared to NK cells from mice vaccinated with a control vaccine. Conclusion: We describe the first known HLA class I restricted tumour associated antigen to be targeted specifically by NK cells. We also demonstrate proof-of-concept for an NK cell targeting peptide vaccination strategy for HCC.

FRI511
Myeloid IRE1a deletion alters hepatic macrophage phenotype and attenuates experimental non-alcoholic steatohepatitis-related hepatocellular carcinoma
Sanne Van Campenhout1, Laurentijn Tilleman2, Sander Lefere1, Astrid Vandierendonck1, Anja Geerts1, Xavier Verhelst1, Filip Van Nieuwerburgh2, Hans Van Vlierberghe3, Lindsey Devisscher4, 1Ghent University, Internal Medicine and Pediatrics, Gent, Belgium; 2Ghent University, Pharmaceutics, Ghent, Belgium; 3Ghent University, Basic and Applied Medical Sciences, Ghent, Belgium
Email: Sanne.VanCampenhout@UGent.be

Background and Aims: Obesity, diabetes and associated non-alcoholic steatohepatitis (NASH) are characterized by adipose tissue and hepatic fat accumulation and inflammation and are rising causes of hepatocellular carcinoma (HCC). Macrophages are important immune cells involved in inflammation and tumour development. Inositol-requiring enzyme 1 alpha (IRE1a) has shown to be involved in macrophage cytokine production and myeloid-specific IRE1a knock-out (mKO) mice showed reduced weight gain during high fat diet feeding. However, the effect of myeloid-specific IRE1a deletion on NASH and subsequent HCC development has not been examined.

Method: Mice with non-functional myeloid IRE1a were created by crossing IRE1a floxed mice with LysM-Cre mice. Two-day old mKO and wild type (WT) mice were subcutaneously injected with streptozotocin (STZ) or PBS as control and male mice were fed a high-fat, -sucrose, -cholesterol diet (Western diet, WD) or control diet from the age of 4 weeks until 21 weeks. Mice were evaluated for obesity, diabetes, NASH and HCC. The macrophage population was evaluated by flow cytometry and RNA sequencing on FACS isolated cells.

Results: STZ+WD feeding resulted in impaired glucose tolerance, advanced NASH with fibrosis and HCC development. mKO STZ mice showed lower fasting glucose levels at the start of WD feeding, and an improved glucose tolerance and attenuated HCC development after 17 weeks of WD feeding despite a similar degree of liver steatosis and inflammation compared to WT mice. Transcriptomic analysis of liver Kupffer cells (KCs), macrophages and monocytes revealed phenotypic changes in NASH-HCC. Myeloid IRE1a deletion in healthy mice resulted in an altered transcriptomic profile with downregulation of pathways involved in immune system activation in KCs and macrophages, downregulation of metabolic pathways in KCs, whereas pathways involved in cell division and metabolism were upregulated in monocytes. Macrophages showed both up- and downregulated metabolic pathways. NASH-HCC attenuated the differential gene expression profile of mKO and WT liver isolated macrophages.

Conclusion: Our results show that myeloid-specific IRE1a deletion results in an altered transcriptional profile of hepatic macrophages and attenuates diabetes induction and NASH-related HCC development.

FRI512
TAK1 is a novel therapeutic target for hepatocellular carcinoma and contributes to sorafenib resistance
Shunjie Xia1, Yu Pan1, Junjie Xu2, Xiujun Cai1, 1Key Laboratory of Laparoscopic Technology of Zhejiang Province, Department of General Surgery, Sir Run–Run Shaw Hospital, Zhejiang University School of Medicine, Hangzhou, China; 2Sir Run–Run Shaw Hospital, Zhejiang University School of Medicine, Department of General Surgery, Hangzhou, China
Email: srrsh_cxj@zju.edu.cn

Background and Aims: TAK1 has a dual role in cancer development and is associated with drug resistance in HCC. The upregulation and activation of TAK1 in intermediate and advanced HCC remains unclear. Mechanistically, little is known about K48-linked ubiquitination and proteasomal degradation of TAK1. This article aims to uncover the mechanism of TAK1 overexpression and its contribution to sorafenib resistance in HCC, and to verify whether targeting TAK1 pharmacologically could be a promising combinational therapy with sorafenib.