Basic Hepatology

IDDF2021-ABS-0073 CHARACTERISTICS OF BILIARY MICROBES IN PATIENTS WITH RECURRENT CHOLEDOCHOLITHIASIS

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10.1136/gutjnl-2021-IDDF.16

Background To observe the characteristics of biliary tract microorganisms in patients with recurrent cholelithiasis, and to analyze the influence of biliary tract microorganisms on the recurrence of cholelithiasis.

Methods The clinical data of 40 patients with recurrent cholelithiasis who completed the operation and one-year follow-up in our hospital from March 2017 to August 2019 were retrospectively analyzed. The clinical data of 40 patients with non-recurrent cholelithiasis who were followed up for 1 year were regarded as the non-recurrent group. Observed and compared the characteristics of biliary tract microorganisms in two groups, and analyzed the effect of biliary tract microorganisms on the recurrence of cholelithiasis.

Results The number of biliary microbial pathogens in the relapsed group and the non-relapsed group were 37 and 33. The number of Gram-negative strains in the relapsed group was the same as that in the non-relapsed group. The relapsed group had more gram-positive bacteria and fungi than the non-relapsed group ($P=0.449$). *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus*, and *Propionibacterium acnes* were higher in the relapsed group than in the non-relapsed group ($P<0.05$); Logistic regression analysis showed that *Escherichia coli*, *Staphylococcus aureus*, intestine Proliferation of cocci and *Propionibacterium acnes* were the influencing factors for recurrent cholelithiasis ($P<0.05$); By drawing ROC curve found that the EUC of *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus*, *Propionibacterium acnes* for the prediction of recurrent common bile duct stones were: 0.813, 0.809, 0.807, 0.806, which have certain predictive value.

Conclusions Patients with recurrent cholelithiasis have significant changes in biliary microorganisms, and the detected pathogenic microorganisms are more significant than non-recurrent ones, such as *Escherichia coli*, *Staphylococcus aureus*, *Enterococcus*, *Propionibacterium acnes*, etc. The proliferation of these pathogenic microorganisms may be related to the occurrence and recurrence of cholelithiasis. It is recommended that patients with cholelithiasis who are treated for the first time can pay close attention to the changes of biliary tract microbes after treatment. For patients who have detected the above pathogenic microorganisms, timely measures should be taken to adjust, which may have a positive meaning in reducing the recurrence of cholelithiasis.

IDDF2021-ABS-0115 ENHANCER DYSREGULATION OF MYELOID-DERIVED SUPPRESSOR CELLS IN HEPATOCELLULAR CARCINOMA

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10.1136/gutjnl-2021-IDDF.17

Background Though immune checkpoint blockade (ICB) therapies have shown promise to treat HCC, the insufficient T cell infiltration to the non-inflamed tumor restricts the effectiveness of ICB therapy to a minority of HCC patients. As a key player in the tumor microenvironment (TME), myeloid-derived suppressor cells (MDSC) were reported to correlate with resistance to ICB and poor prognosis. Given the fact that enhancer reprogramming of MDSC is crucial to the MDSC identity and function, this study aims to identify the enhancer targets that are significantly contributing to MDSC immunosuppressiveness and to study the potential of myeloid targeting for improving immunotherapy efficacy.

Methods Single-cell RNA sequencing (sc-RNA-seq) of ICB-resistant patients was performed to understand the immune profile of patients and the heterogeneity of TME in HCC. The expression and immune profile of MDSC signature genes were determined by flow cytometry analysis in immune cells from tumor-bearing mice and HCC patients. FANTOM5 (Functional Annotation of the Mammalian Genome) database and JEME (Joint Effects of Multiple Enhancers) algorithm were used to identify enhancer RNA (eRNA) locations and expressions. Functional significance and molecular mechanisms of signature genes were conducted by gene knockdown in human blood-derived MDSCs, followed by mRNA and protein detection, q-ChIP-PCR and multi-colour flow cytometry.

Results MDSCs were negatively correlated with $CD8^+$ T cell proportion in ICB-resistant patients. A novel MDSC signature gene, EREG has been identified. Its expression is correlated with non-responsiveness in HCC patients. It is enriched in MDSCs of HCC patients and correlated with poor prognosis. Moreover, high EREG expression is correlated with increased tumor size and decreased tumor-infiltrating lymphocytes in ICB resistance mouse models. Mechanistically, it was found that eRNAs control the upregulation of EREG in MDSCs. Inhibition of eRNAs could reduce MDSC proliferation and T cell suppressive activity.

Conclusions Our data demonstrated the intricate interaction of enhancer regulation of EREG in MDSC and ICB resistance, delineating a new epigenetic mechanism underlying tumor immune evasion. Identifying this novel enhancer-regulated target might uncover new immunosuppressive mechanism and MDSC-directed strategy for improving HCC immunotherapy efficacy.

IDDF2021-ABS-0138 CIRCULATING NON-CODING TRANSCRIPTS SERVING AS BIOMARKERS FOR DIABETIC LIVER STEATOSIS

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10.1136/gutjnl-2021-IDDF.18

Background Non-alcoholic steatohepatitis (NASH) as an advanced form of NAFLD is associated with excessive inflammation in the steatotic livers. Given the common comorbidity of NASH with diabetes mellitus (DM), we identified a set of circulating RNA transcripts as non-invasive

biomarkers to classify disease phenotypes relevant to the onset of diabetic steatosis followed by progression towards liver inflammation.

Methods We compared miR-192-3p level in mice given HFD (high-fat diet), HFFD (high-fat fructose diet), and MCD (methionine-choline deficient diet) diets. Histopathological lesions were analyzed at critical stages to correlate the miRNA expression. In obese individuals diagnosed with DM or not, we examined the miR-192-3p level by droplet digital PCR and characterized serum whole transcriptomes with SEQuoia Complete Stranded RNA Library kits.

Results Along with our reported loss of the overlooked star strand miR-192-3p in db/db mice, we found a high circulating level of miR-192-3p in different diets-induced animals, indicating a heightened inflammatory response in the liver, especially in those given HFFD and MCD diets. Since circulating miR-192-3p surges only in HFFD under an insulin-resistant state but not manifested at all in HFD, its circulating level shall reflect the extent of runaway glycerolipid metabolism. Indeed, we observed a similar expression pattern in serum miR-192-3p for obese individuals (BMI>34), where the miRNA can only be detected in individuals diagnosed with DM. Our transcriptomic analysis identified that PI3K-Akt signaling, FoxO signaling and TGF β signaling pathways were enriched in the hepatocytes with miR-192-3p overexpression. The top differential circulating non-coding transcripts between those individuals with or without diabetes belonged to the ribosomal pathway under the modulating actions of Akt, suggesting the use of these transcripts for a better account of diabetic phenotype.

Conclusions Circulating levels of miR-192-3p and transcripts along its regulated Akt pathway account for visceral obesity and reflect prognostic outcomes to the progression of diabetes. We hereby advocate miR-192-3p as a functional glycerolipid regulator for steatosis in the diabetic liver which offers know-how for better disease staging and treatment regimes.

IDDF2021-ABS-0204 SARS-COV-2 PRODUCTIVELY INFECTS HUMAN LIVER AND BILIARY ORGANIDS

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10.1136/gutjnl-2021-IDDF.19

Background SARS-CoV-2 has caused the COVID-19 pandemic, 14-53% with COVID-19 developed hepatic dysfunction. More data is required to ascertain the pattern of liver injury in patients with COVID-19. Here we report the use of human liver and biliary organoids as a tool to investigate the SARS-CoV-2 infection and virus-induced tissue damage ex vivo at the cellular and molecular levels.

Methods Immunofluorescence staining and RT-qPCR was performed to examine the susceptibility and replication of human liver and biliary organoids to SARS-CoV-2. Transmission electron microscopy was performed to investigate the ultrastructure of SARS-CoV-2 infected biliary organoid. The mRNA sequence analysis was performed to determine gene expression changes induced by SARS-CoV-2-infection of biliary organoids.

Results Immunofluorescence staining showed that SARS-CoV-2 spike (S) glycoprotein protein was readily detected in patchy areas of the human liver and biliary organoids, whereas no signal was found in uninfected control. RT-qPCR showed that the SARS-CoV-2 genomic RNAs revealed a dramatic increase of viral load in organoids at 24 h post-infection (MOI=1) and it can remain stable 96 hours in the liver organoids and 48 hours in biliary organoids. Unsupervised transmission electron microscopy (TEM) showed that viral particles occurred in the lumen of the organoid, at the basolateral and apical side of the organoid, even in membrane-bound vesicles. Additionally, RNA-seq revealed early cell response to virus infection, including the well-known upregulation of chemokines and inflammatory cytokines.

Conclusions Therefore, the hepatocytes and bile duct cells have effectively been infected by COVID-19, and human liver and biliary organoids can serve as a pathophysiological model to investigate the underlying mechanism of SARS-CoV-2 infection.

IDDF2021-ABS-0207 TYPE 2 RESISTANT STARCH IMPROVES LIVER STEATOSIS INDUCED BY HIGH-FAT DIET RELATING TO GUT MICROBIOTA REGULATION AND CONCENTRATION OF PROPIONIC ACID IN PORTAL VEIN BLOOD IN C57BL/6J MICE

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10.1136/gutjnl-2021-IDDF.20

Background Resistant starch (RS) was approved to regulate glucose and lipid metabolism and gut microbiota in vivo. Our aim was to explore the effect and mechanism of type 2 resistant starch (RS2) on NAFLD mice.

Methods Twenty C57BL/6J male mice were randomly divided into CD group, CD+RS group, HFD group, HFD+RS group (n=5). The NAFLD mouse model was induced by a high-fat diet for 16 weeks. RS2 was supplemented into the diet for 4 weeks subsequently. After treatment, liver steatosis was evaluated by HE staining and NAS score. The serum alanine aminotransferase (ALT), triglyceride (TG), fasting serum glucose (FBG) and insulin resistance index (HOMA-IR) of portal vein blood were detected. The intestinal content of mice was collected for microbiota analysis by 16s amplicon sequencing. The level of short-chain fatty acids (SCFA) in portal vein blood was detected.

Results Compared with HFD group, after RS intervention, the liver steatosis of mice was significantly reduced, and the serum levels of ALT, TG, FBG and HOMA-IR were significantly decreased ($P<0.05$) (IDDF2021-ABS-0207 Figure 1a Type 2 resistant starch improves liver steatosis induced by high-fat diet; Hepatic HE staining of mice) (IDDF2021-ABS-0207 Figure 1b Type 2 resistant starch improves liver steatosis induced by high-fat diet; NAS score of mice (** $P<0.01$)) (IDDF2021-ABS-0207 Figure 1c Type 2 resistant starch improves liver steatosis induced by high-fat diet; Serum levels of ALT in mice (** $P<0.01$) (IDDF2021-ABS-