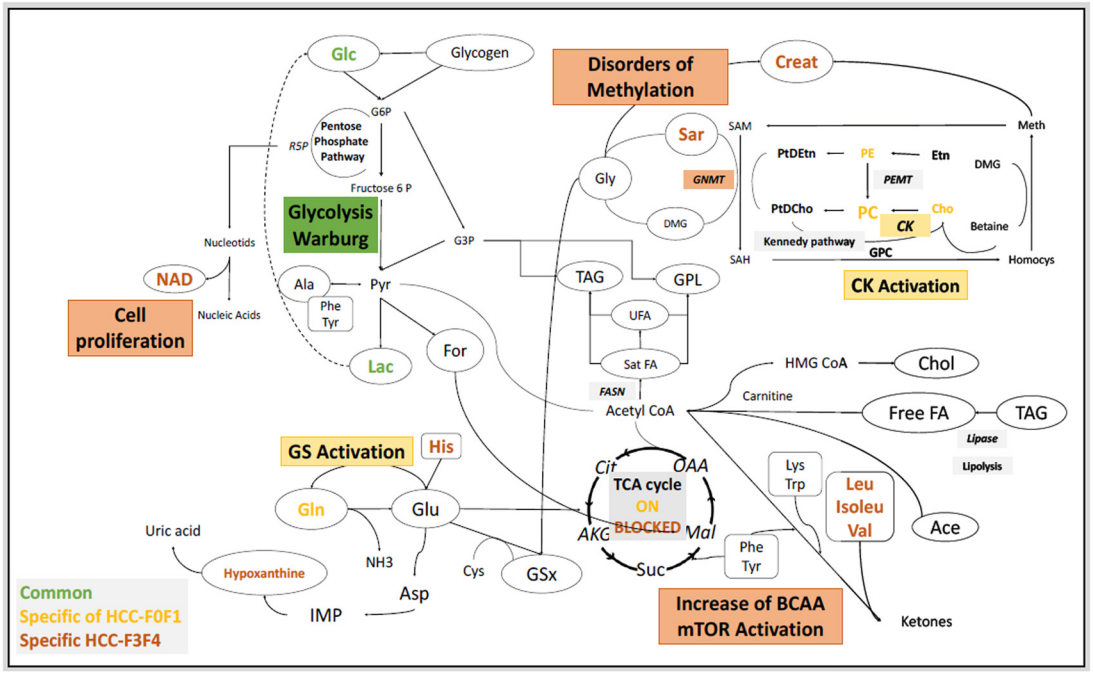

Figure: (abstract: THU-455): Hypothetic mechanistic pathways of HCC-NAFLD according to fibrosis severity.

well as an increase of saturated fatty acid levels suggesting an up-regulation of fatty acid synthase activity (FASN) which may contribute to tumor growth and proliferation.

Conclusion: In conclusion, metabolomic analysis of aqueous and lipid extracts allow us to suggest for the first time that there are two phenotypes of HCC developed in NAFLD according to the fibrosis level. This study highlights the impact of underlying pathology on metabolic reprogramming of the tumor.

THU-456

Polyploidy spectrum: a new marker of molecular HCC tumour classification

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Background and aims: Polyploidy is a fascinating characteristic of liver parenchyma. Hepatocytes polyploidy depends on the DNA content of each nucleus (nuclear ploidy) plus the number of nuclei per cell (cellular ploidy). Which role can be assigned to polyploidy during human HCC development is still an open question. Here, we investigated whether a specific ploidy spectrum is associated with clinical and molecular features of HCC.

Method: Ploidy spectra were determined on a total of 85 paired (tumor/adjacent) surgically resected tissues from HCC patients as well as 5 tissues from healthy control. To define ploidy profiles, quantitative and qualitative *in situ* imaging approach was used on paraffin tissue liver sections.

Results: We first demonstrated that polyploid hepatocytes are major components of human liver parenchyma, polyploidy being mainly cellular (binuclear hepatocytes). Across liver lobules, polyploid hepatocytes do not exhibit specific zonation pattern. During liver tumorigenesis, cellular ploidy is drastically reduced; binuclear polyploid hepatocytes being barely present in in HCC tumors. Remarkably, nuclear ploidy is specifically amplified in HCC tumors. In fact, nuclear ploidy is more amplified in HCCs harboring low grade of differentiation and related to p53 mutations. Our results finally demonstrated that highly polyploid tumors are associated with poor prognosis and higher proliferation rate.

Conclusion: Our results underscore the importance of quantification of cellular and nuclear ploidy spectrums as an accurate diagnostic test for HCC tumorigenesis.

THU-457

DoubleCortin/Like Kinase 1 (DCLK1) expression characterized specific cancer stem cell subpopulations of human cholangiocarcinoma primary cell cultures where its inhibition exerts anti-neoplastic effects

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Background and aims: Cholangiocarcinoma (CCA) is a very aggressive cancer with high chemoresistance. We demonstrated that CCA is enriched of Cancer Stem Cells (CSCs); this feature is associated with aggressiveness and drug resistance. Recently, DCLK1 was validated as a CSC marker in different gastrointestinal tumors.
Our aims were to evaluate: i) DCLK1 expression and biological function in primary cell cultures of CCA subtypes iCCA (intrahepatic) and pCCA (perihilar) and; ii) DCLK1 expression in human CCA samples *in situ* and its serum concentration.
Method: Primary cell cultures were prepared from surgical specimens of human iCCA and pCCA and CSCs were immunosorted for specific markers (LGR5, CD13, CD90, EpCAM, CD133). hBTSC and hHPSC physiological primary stem cell cultures were used as controls of iCCA and pCCA respectively. DCLK1 expression was analysed by RT-qPCR, western blot and immunofluorescence. In functional studies, the effects of a selective DCLK1 inhibitor (LRRK2-IN-1, 72hrs of treatment) on cell proliferation (MTS Assay, population doubling time-PDT), apoptosis (AnnessinV-FITC/PI) and colony formation capacity were evaluated. DCLK1 gene expression in surgical resected CCA and healthy samples was evaluated by RT-qPCR. DCLK1 serum concentration was measured in CCA, HCC, cirrhotic and healthy patients by ELISA.
Results: For the first time, we demonstrated DCLK1 mRNA and protein expression in iCCA and pCCA. An increased expression of DCLK1 in CCA was evidenced in association with other CSC markers and its highest expression was observed in specific subpopulations of CCA-CSCs (i.e. $pCCA^{LGR5+}$ and $iCCA^{CD133+}$). DCLK1 showed cytoplasmic localization in $pCCA^{LGR5+}$, $iCCA^{CD133+}$, unsorted pCCA and iCCA cell cultures. LRRK2-IN-1 (5 μM) added to CCA cultures increased PDT, decreased proliferation, colony formation capacity and colony size, and induced apoptosis in both iCCA and pCCA compared with controls ($p < 0.01$). LRRK2-IN-1 showed a dose-dependent anti-proliferative effect (2.5μM-20 μM) by MTS assay with an IC_{50} of 9.61 μM in unsorted pCCA, 14.72 μM in unsorted iCCA, 4.51 μM in $pCCA^{LGR5+}$ and 9.61 μM in $iCCA^{CD133+}$ cells. Furthermore, LRRK2-IN-1 did not influence hBTSC and hHPSC primary cell cultures viability. DCLK1 gene expression was lower in healthy tissues than in specimens of iCCA and pCCA ($p < 0.01$). Interestingly, DCLK1 was detected in serum samples of iCCA and pCCA patients. DCLK1 serum levels were lower in cirrhotic and HCC patients compared to CCA patients ($p < 0.05$), but we have never observed DCLK1 protein into serum samples of healthy controls.
Conclusion: In conclusion, DCLK1 expression characterizes specific CCA-CSC subpopulations and could represent a serum biomarker for CCA. DCLK1 inhibition exerts anti-neoplastic effects in primary CCA cell cultures.

THU-458

Identification of relevant oncogenes in RAS-activated primary liver cancer using a targeted screening approach

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Background and aims: Gene mutations within the RAS signaling pathway occur with a frequency of 15–20% in human hepatocellular carcinoma (HCC) and intrahepatic cholangiocarcinoma (ICC). Activation of RAS signaling is associated with a poor prognosis. However, it remains unclear if specific targeted therapies could be beneficial in this subgroup of patients. In the RPK mouse model, liver-specific expression of oncogenic *Kras* and genetic inactivation of the tumor suppressors *Rb* and p53 mimics genetic changes found in a subgroup of human primary liver cancers. Hepatic tumors in this model resemble human HCC and ICC, as well as tumors of mixed HCC/ICC differentiation. We used a targeted *in vitro*-screening approach to identify relevant oncogenes in our model, followed by *in vivo*-transfection of mouse hepatocytes with transposon-based shRNA constructs.
Method: RNA and protein from microdissected liver tumors of $Rb^{lox/lox};p53^{lox/lox};Kras^{LSL-G12D/+}$ (*RPK*) mice and liver tissue of age matched control mice were analyzed by mRNA microarray, qPCR and western blot. Gene knock-down in primary cell lines from RPK tumors was achieved using lentiviral *shRNA* constructs. Proliferation and clonogenic assays were used to test for the functional relevance of target genes. Transposon *CreER*-constructs harboring inducible *shRNAs* were injected by hydrodynamic tail vein injection to achieve target gene knock-down *in vivo*.
Results: Highly upregulated genes in *RPK* tumors were identified by mRNA microarray analysis. Of all genes tested, only shRNA constructs against *Dmbt1* significantly decreased cell proliferation and clonogenic capacity. Knock-down of *Dmbt1* was verified by mRNA and protein analysis. *In vivo* experiments using *CreER-shDmbt1* transposon constructs showed a significantly longer survival of RPK mice in comparison to controls injected with a random shRNA construct.
Conclusion: Activation of RAS signaling is found in up to one in five patients with primary liver cancer and is associated with a poor prognosis. Using a targeted screening approach in a mouse model with RAS-dependent liver tumors, we identified *Dmbt1* as a relevant oncogene. Of note, DMBT1 functions as a tumor suppressor in other cancers probably through suppression of anti-cancer immunity. Here, we identified a novel oncogenic role of *Dmbt1* in the liver cancer. Interestingly, *DMBT1* is upregulated in a subset of human HCC and many ICC, where it could present a possible target for cancer therapy.

THU-459

Non-parenchymal TREM2 halts hepatocarcinogenesis through the inhibition of liver inflammation and hepatocyte proliferation

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Background and aims: Hepatocellular carcinoma (HCC) is a prevalent and aggressive cancer that usually arises on a background of chronic liver injury where liver regenerative and inflammatory processes are involved. The triggering receptor expressed on myeloid cells 2 (TREM2) is predominantly expressed in hepatic non-parenchymal cells and inhibits Toll-like receptor (TLR)-derived signalling, protecting the liver from various types of hepatotoxic injury. However, its role in liver cancer is unknown. Here, the role of