Applied Medical Research, University of Navarra, Pamplona; [4]Liver and Gastrointestinal Diseases, Biodonostia Research Institute, Donostia University Hospital, University of the Basque Country, CIBERehd, Ikerbasque, San Sebastian; [5]Hematology and Cell Therapy Area, Centre for Applied Medical Research, Clinic University of Navarra; [6]Centro de Investigación Biomédica en Red (CIBERehd), Clinic University of Navarra, Pamplona, Spain
E-mail: mbarcena.1@alumni.unav.es

Background and Aims: Hepatocellular carcinoma (HCC) incidence keeps increasing and patients' survival remains very low. In most cases HCCs appear on chronically inflamed and cirrhotic livers. Despite all the progress in understanding the cellular and molecular mechanisms of liver fibrosis and carcinogenesis, there are no effective therapies to halt fibrosis or quell liver cancer. We have analyzed in detail the role in liver tumor pathology of the H3K9 histone methyltransferase G9a together with DNA methyltransferase 1 (DNMT1). These two epigenetic modifiers are known to physically and functionally interact in the regulation of gene expression, contributing to the growth of different types of tumors. We also describe first-in-class substrate-competitive dual inhibitors of G9a and DNMT1, and explore their antitumoral efficacy in different in vitro and in vivo models of HCC.
Results: G9a expression is significantly up-regulated in human HCCs, mouse models of fibrosis-associated HCC and in human HCC cell lines compared to normal hepatocytes. G9a is also markedly upregulated during mouse hepatic stellate cells (HSCs) activation in culture. siRNA-mediated G9a and DNMT1 knockdown markedly inhibited HCC cells growth and survival, as well as the fibrogenic activation of HSC. Our G9a/DNMT1 dual inhibitors showed a high degree of specificity when tested against 32 different epigenetic modifiers in vitro, and GI50 values in the nM range towards HCC cell lines. CM272, one of the best performing inhibitors analyzed, presented a good safety profile in vivo, and potent antifibrotic and antitumoral properties. These were demonstrated in vitro and in several in vivo models of liver fibrosis and HCC, including subcutaneous xenografts of mixed liver fibroblasts and HCC cells as well as orthotopic xenografts. Mechanistically, CM272 interfered with the entire process of glycolytic metabolic reprogramming involved in HCC cells growth and in TGFb-induced fibrogenic activation of HSC cells. Inhibition of G9a and DNMT1 in these cells completely altered three glycolytic shunt pathways: the pentose phosphate pathway, the serine-glycine synthesis pathway and one-carbon metabolism. This resulted in an inefficient adaptive response to hypoxia, the single most important feature of the microenvironment driving HCC progression.
Conclusions: Our study suggests that the pharmacologic interference with G9a and DNMT1 may be a novel and promising strategy for the development of effective therapies in fibrosis-associated HCC.

PS-140
Tumor derived exsomes mediate tumor infiltrating NK-cell dysfunction in patients with hepatocellular carcinoma via TGF-β/SMAD pathway

Q.-F. Zhang[1], W.-W. Yin[1], Q. Liu[1], Y.-L. Li[1], M.-H. Li[1], J.-Y. Shao[1], Q.-F. He[1], H.-D. Hu[1], P. Hu[1], H. Ren[1], D.-Z. Zhang[1]. *[1]Key Laboratory of Molecular Biology for Infectious Diseases (Ministry of Education), Department of Infectious Diseases, Institute for Viral Hepatitis, The Second Affiliated Hospital, Chongqing Medical University, Chongqing, China*
E-mail: dzhzhang@yahoo.com

Background and Aims: Natural killer (NK) cells play a vital role in killing hepatocellular carcinoma (HCC) cells and defects in NK cell-function are necessary for tumor immune escape. Emerging studies on tumor cell-derived exosomes (Texs) have shown the biological significance in tumor development and microenvironment, but the underlying role of Texs in regulating NK-cell dysfunctions in HCC patients remains largely unknown.
Methods: Flow cytometry staining was used to detect the phenotype and function of NK cells in 36 HCC patients VS 36 healthy controls (HCs). Transmission electron microscopy and Western blotting experiments were performed to characterize Texs.
Results: Firstly, we precisely characterized the phenotype and function of NK cells in HCC patients VS HCs. With an inhibitory phenotype, tumor-infiltrating NK (TINK) cells exhibited poor cytotoxic capacity and deficient potential to produce IFN-γ compared with NK cells from tumor margin tissue and nontumorous tissue. Next, we revealed that HCC cells trigged NK-cell dysfunction by a exosome-dependent mechanism. Interestingly, HCC cell-derived exsomes were preferentially enriched with transforming growth factor-beta1 (TGF-β1), which acted as important mediators of TINK-cell functional deficiency. The TGF-β/SMAD signaling pathway was constitutively activated in TINK cells from HCC patients when compared to NK cells from tumor margin tissue and nontumorous tissue. Culture in vitro of HCC cells or Texs with healthy NK cells induced NK dysfunction mediated by activation of the TGF-β/SMAD signaling pathway, and abrogated by blocking TGF-β.
Conclusions: These data indicate that by regulating the TGF-β/SMAD pathway, Texs induce TINK-cell dysfunction to evade innate immune surveillance, thus highlighting the importance of developing novel therapies to target this inhibitory pathway and restore antitumor cytotoxicity.

PS-141
Hepatic LGR5 stem cells contribute to liver carcinogenesis

W. Cao[1], M. Li[1], P. Liu[1], J. Liu[1], M. Bolkestein[2,3], K. Chen[1,4], L.J.W. Van Der Laan[3], D. Sprengers[1], H.J. Metselaar[1], J. Kwekkeboom[1], R. Smits[1], M.P. Peppelenbosch[1], Q. Pan[1]. *[1]Department of Gastroenterology and Hepatology; [2]Department of Cell Biology; [3]Department of Surgery, Erasmus Medical Center, Rotterdam, The Netherlands; [4]College of Life Sciences, Zhejiang Sci-Tech University, Hangzhou, China*
E-mail: w.cao@erasmusmc.nl

Background and Aims: The concept that adult stem cells can accumulate genetic/epigenetic changes and subsequently contribute to tumor initiation and progression has attracted great interest, but remains controversial. Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5) is a recently identified marker for a liver stem cell population. Here we investigate the role of hepatic LGR5-expressing cells in liver carcinogenesis.
Methods: A LGR5-promotor driven diphtheria toxin (DT) receptor knock-in mice model with a GFP reporter and a lineage tracing mice model with a membrane-targeted tandem dimer Tomato/green fluorescent protein (mTmG) reporter were used. Carbon tetrachloride (CCl_4) was used to induce chronic liver injury and diethylnitrosamine (DEN) was used to induce primary liver tumor in mice.
Results: We observe the absence of a LGR5-expressing compartment in the mouse liver throughout an unchallenged life span, but it is induced upon CCl_4-induced injury. However, this liver LGR5-positive compartment has only limited contribution to tissue repair as observed by lineage tracing. Surprisingly, we find that the carcinogen DEN also induces a liver LGR5-positive stem cell compartment. In thus-induced hepatic tumors, the percentage of LGR5 cells is significantly higher as compared to tumor adjacent tissue ($n = 28$, $P < 0.0001$, 4-fold higher), and this even more apparent when contrasted to tissue of CCl_4-induced chronic injury ($n = 28$, $P < 0.0001$, 66-fold higher). Tumor organoids generated by *ex vivo* culturing of primary mouse liver cancer contain a LGR5-expressing cell population. Subcutaneous transplantation of these tumor organoids into immunodeficient NOG mice results in solid tumors, which retain a LGR5 positive compartment. Isolation and culturing of single $LGR5^+$ cell from primary mouse tumor initiated tumor organoids, and transplantation of these organoids into NOG mice formed tumor again. Thus, these cells have cancer initiating/stem cell-like properties. Importantly, lineage tracing shows that liver $LGR5^+$ stem cells and their daughters cells contribute to the development of liver tumor.

Conclusions: Hepatic LGR5 stem cells are only induced following liver injury and importantly contribute to DEN-induced liver carcinogenesis but not to tissue repair. Thus targeting LGR5-positive cells appears promising as an anti-cancer strategy in the liver.

PS-142

Immune gene expression profile in hepatocellular carcinoma and surrounding tissue predicts time to tumor recurrence

C. Carone[1], A. Olivani[2], R.D. Valle[3], T. Trenti[1], G. Missale[2], E. Cariani[1].
[1]*Toxicology and Advanced Diagnostics, Ospedale S. Agostino-Estense, Modena;* [2]*U.O. Infectious Diseases and Hepatology, Azienda Ospedaliero-Universitaria di Parma;* [3]*Department of Surgery, University of Parma, Parma, Italy*
E-mail: ecariani@hotmail.com

Background and Aims: The anti-tumor immune response may play a major role on the clinical outcome of hepatocellular carcinoma (HCC). We characterized the liver immune microenvironment by direct hybridization of RNA extracted from HCC and non-tumorous tissues.
Methods: RNA was extracted from frozen liver tissue samples of HCC (T, n. 30) and non-tumorous tissues (NT, n. 33) obtained from 38 patients. Matched samples were available for 25 patients. The immune gene expression profile was analyzed by the nCounter GX Human Immunology v2 system (NanoString Technologies) that detects the expression levels of 579 immune response-related genes simultaneously.
Results: The immune gene expression profile of T and NT tissues was significantly different ($p < 0.05$). The possible prognostic relevance of liver immune microenvironment was therefore evaluated in T and NT samples separately. Unsupervised clustering detected two main clusters of immune gene expression both in T and in NT liver samples. In both cases, expression clusters identified groups of patients with significantly different median time to HCC recurrence. Based on T tissue, two groups with median TTR of 18.5 and 127 months, respectively, were detected ($p = 0.005$). Expression of genes related to inflammation, T and B cell activation were associated with longer TTR. The analysis of NT tissues discriminated subsets of patients with median TTR of 19 and 68 months ($p = 0.032$). By contrast to T tissue, a predominant inflammatory immune environment was associated with shorter TTR. The liver immune gene profile was not statistically related to overall survival in this series.
Conclusions: We evaluated immune gene expression by direct hybridization of RNA extracted from liver tissue. Immune gene expression profiles predictive of TTR could be identified both in HCC and in adjacent cirrhotic tissues. Longer TTR was associated with overexpression in T tissue and downregulation in NT tissue of inflammation-related genes.

PS-143

Chk2 DNA damage response protein mislocalization further enhances chromosomal instability and human hepatocellular carcinoma progression

V. Carloni[1], M. Lulli[2], S. Madiai[1], T. Mello[3], A. Hall[4], T.V. Luong[4], M. Pinzani[5], K. Rombouts[5], A.G. Galli[3]. [1]*Department of Experimental and Clinical Medicine;* [2]*Department of Experimental and Clinical Biomedical Sciences, Pathology Unit;* [3]*Department of Experimental and Clinical Biochemical Sciences, Gastroenterology Unit, University of Florence, Florence, Italy;* [4]*Department of Cellular Pathology, Royal Free Hospital;* [5]*Division of Medicine, Institute for Liver &Digestive Health, University College London (UCL), London, United Kingdom*
E-mail: k.rombouts@ucl.ac.uk

Background and Aims: High levels of genomic instability correlate with progression in hepatocellular carcinoma (HCC) of which the most common form is chromosomal instability (CIN), resulting in heterogeneity, with drug resistance and immunity escape as a consequence. CIN *perse* is an important factor of DNA damage sustaining numerical/structural chromosome abnormalities but the underlying mechanisms are not well characterized. In this study, the role of Chk2, a DNA damage response kinase was investigated.
Methods: An animal model of diethylnitrosamine-induced HCC was employed known to induce DNA damage and elevated mitotic errors. DNA damage response kinase Chk2 localization was determined in two cohorts of human HCC specimens. To assess the functional role of Chk2, gain on-and loss-of-function, mutation analysis with Chk2 variants, karyotyping, immunofluorescence/live imaging were performed. Three different cell lines were used: HCT116, a near-diploid cell line, Huh7 a stable hyperdiploid karyotype, and human hepatocytes (HuS) immortalized with *TERT* gene.
Results: Tumours of DEN-treated animals showed nuclear upregulation of Chk2 and P-H2A.X known to be activated in the presence of DNA damage. *In vitro*, defective chromosome segregations caused DNA damage *per se* and induced Chk2 overexpression. Chk2 overexpression/phosphorylation-activation and mislocalization occurred exclusively within mitotic components of HuS30gen. This coincided with an increased mitotic index which was reversed by knockdown of Chk2. The forkhead-associated (FHA) domain of Chk2 is uniquely essential for proper localization to mitotic structures. Aurora B kinase and P-Histone H3 colocalized with Chk2 when mislocalized, thus sustaining a constant mitotic activity. Retinoblastoma phosphorylation contributed to defective mitoses, but not p53. Chk2 expression was investigated in two cohorts of HCC tissues. A strong cytoplasmic and perinuclear presence in Grade I and Grade II was observed. In contrast, grade III HCC tissues were marked by a strong and exclusive nuclear Chk2. RNA-Seq-based transcriptomes of 188 HCC tissues (TCGA) were analysed; 102 with TP53 mutations and 86 with CNNB1 mutations. Chk2 was identified to be significantly associated with HCC TP53 mutations characterized by CIN.
Conclusions: The study reveals a new mechanistic insight in the co-involvement of Chk2 in HCC progression. These findings propose Chk2 as a putative biomarker to detect CIN in HCC providing a valuable support for clinical/therapeutical management of patients.

PS-144

Diabetes augments obesity in accelerating liver tumour development: role of oxidative stress-induced JNK signalling and DNA damage response

E. Arfianti[1,2], V. Barn[1], S. Pok[1], C.Z. Larter[1], N.C. Teoh[1], G.C. Farrell[1].
[1]*Liver Research Group, The Australian National University, Canberra, Australia;* [2]*Department of Medical Biology, Riau University, Pekanbaru, Indonesia*
E-mail: arfianti.arfianti@anu.edu.au

Background and Aims: Obesity and diabetes are independent risk factors for hepatocellular carcinoma (HCC) yet the contribution of each metabolic condition has not been clarified. Earlier, we demonstrated that obese diabetic *foz/foz* mice exhibit accelerated diethylnitrosamine (DEN)-induced HCC. To establish whether obesity itself or diabetes is more relevant to enhanced HCC development, we compared chemically induced HCC between equally obese diabetic *Alms1* mutant (*foz/foz*) NOD.B10 and non-diabetic *foz/foz* BALB/c mice.
Methods: Male *foz/foz* and *Wt* NOD.B10 and BALB/c mice were injected with DEN (10 mg/kg) at 12–15 days, controls with saline. Hepatic protein expression was assayed by immunoblotting and immunohistochemistry.
Results: Both strains of *foz/foz* mice developed equivalent obesity, but metabolic complications of obesity, including hepatomegaly, insulin resistance with hyperinsulinemia, and hyperglycemia, occurred only in *foz/foz* NOD.B10 mice. At 6 mths, the incidence of liver tumours was significantly higher in *foz/foz* NOD.B10 than *foz/foz* BALB/c mice (100% vs 40%). Liver nodules were also more