baseline and HGF-induced FAK, ERK and STAT3 pathway activation. Increasing stiffness results in upregulation of mesenchymal markers (including N-cadherin and vimentin), consistent with mesenchymal shift, and down-regulation of differentiated hepatocyte markers (including albumin, α-1-antitrypsin and HNF4). Following treatment with cisplatin, cells cultured on soft supports were more susceptible to apoptosis (PARP/ Caspase-3 cleavage). However, in both Huh7 and HepG2 cells, surviving cells from soft supports had 2.2-fold ($p<0.05$) and 2.4-fold ($p<0.001$) higher clonogenic capacity respectively, than surviving cells from stiff supports. This was associated with upregulation of cancer stem cell markers (Oct4, NANOG, CD44, CD133, c-kit and CXCR4).

Conclusion HCC is a tumour that develops within an altered biomechanical niche. Increasing matrix stiffness regulates HCC mitogenic signalling, proliferation, differentiation and chemotherapeutic resistance. However, a soft microenvironment (as may be encountered by disseminated tumour cells) promotes stem cell characteristics following chemotherapy. This provides a possible explanation for the failure of systemic chemotherapy both in relation to treatment of primary HCCs and the eradication of disseminated tumour cells that give rise to metastases. The selective targeting of the cytoskeleton represents a potentially novel approach to the treatment of HCC.

P39 ABSTRACT WITHDRAWN

P40 EX VIVO TREATMENT OF NEUTROPHILS WITH A P38-MAPK AGONIST IN PATIENTS WITH LIVER FAILURE IMPROVES THEIR BACTERIOCIDAL CAPACITY

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Introduction Ammonia reduces neutrophil phagocytic and bacteriocidal capacity. Neutrophils swell in response to ammonia exposure and activation of the p38-MAPK pathway has been implicated in protecting the neutrophil against osmotic shock and in normalising neutrophil phagocytic dysfunction. (Shawcross *et al.*, *Hepatology* 2008) In patients with acute and chronic liver failure with elevated arterial ammonia, we hypothesised that treating neutrophils ex vivo with isoproterenol, a β adrenergic receptor and p38-MAPK agonist would improve neutrophil phagocytic capacity.

Aim Within the context of an ongoing longitudinal study of neutrophil function in 100 patients with acute (ALF) and chronic liver failure we investigated the role of ex vivo incubation of neutrophils with an exogenous ammonia load, a p38-MAPK agonist (isoproterenol) and the specific p38-MAPK antagonist SB203580 (Calbiochem) on neutrophil function in a cohort of 10 patients (5 ALF and 5 chronic) all with elevated arterial ammonia concentration.

Method Phagocytic capacity in heparinised whole blood was quantitatively determined by flow cytometry using FITC-labelled opsonised *E. coli* at baseline, and following incubation for 90 min with ammonium chloride (200 μmol/l), isoproterenol (2 μmol/l) or SB203580 (40 μmol/l) at 37°C.

Results The ALF patients (drug-induced n=3 and seronegative n=2) had a median SOFA score of 17 (IQR 16–18) and APACHE II score of 24 (22–28). The patients with cirrhosis (alcohol n=3; HCV; NASH) had a median MELD score of 10 (7–27). The median arterial ammonia level in the ALF group was 96 (49–158) and in the chronic group was 97 (63–122) μmol/l. Baseline bactericidal capacity was 49% (36–57) in the ALF group and 75% (67–84) in the chronic group compared to >85% in healthy controls and a median of 82% in a septic control group without liver disease. The exogenous ammonia load further reduced neutrophil phagocytic capacity by a median of 8% (3–16). The p38-MAPK agonist significantly abrogated the ammonia-induced phagocytic impairment and improved bacteriocidal capacity ($p=0.003$). The p38-MAPK antagonist however, exacerbated the ammonia-induced reduction in neutrophil phagocytic capacity by a median of 15% (4–24); $p=0.0015$.

Conclusion These data show that in patients with acute and chronic liver failure and elevated arterial ammonia that neutrophil bacteriocidal capacity is disabled. Activation of the p38-MAPK pathway serves as an important cellular protective mechanism against ammonia-induced impairment and further trials of p38-MAPK agonists in patients with liver failure are warranted.

P41 GENETIC VARIATION IN BILIARY TRANSPORTERS AS A SUSCEPTIBILITY FACTOR FOR CHOLANGIOCARCINOMA

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Introduction Cholangiocarcinoma (CC) is increasing in incidence globally but its pathogenesis remains poorly understood. Chronic inflammation of the bile duct and cholestasis are major risk factors but most cases in the West are sporadic. Genetic polymorphisms in biliary transporter proteins have been implicated in benign biliary disease and, in the case of progressive familial cholestasis, have been associated with childhood onset of CC. A recent case-control study of a single nucleotide polymorphism c.3972C>T (rs3740066) in ABCC2, reported an association with CC.

Aim To investigate five biologically plausible candidate genes as susceptibility factors for cholangiocarcinoma; ABCB11 (BSEP); ABCB4 (MDR3); ABCC2 (MRP2); ATP8B1 (FIC1) and NR1H4 (FXR).

Method Germline DNA was collected from 172 Caucasian individuals with confirmed CC. A control cohort of 256 healthy Caucasian patients was included in the analysis. 73 SNPs were selected using the HapMap database in Haploview 4.1 (build 22; MAF >0.05, pair-wise comparisons only) to capture the majority of common genetic variation around the five candidate loci. Genotyping was undertaken with a competitive allele-specific PCR based robotic genotyping system. Confirmation of Hardy-Weinberg equilibrium and Cochran-Armitage trend testing were performed using PLINK v1.07. Haplotype frequencies were compared using haplo.stats v1.4.4.

Results All 73 SNPs were in Hardy-Weinberg Equilibrium. Four SNPs in ABCB11 were associated with altered susceptibility to CC, including the V444A polymorphism (c.1331T>C, rs2287622, $p<0.007$) but these associations did not retain statistical significance after Bonferroni correction for multiple testing. Haplotype analysis of the genotyped SNPs in ATP8B1 identified significant differences in frequencies between cases and controls (global p value 0.005). None of the SNPs in ABCC2, including rs3 740 066, showed association with CC. Haplotype analysis in ABCC2 failed to detect significant association.

Conclusion This is the largest study to date of biliary transporter polymorphisms as susceptibility factors for CC. The previously reported association between SNP rs3 740 066 in ABCC2 and CC was not replicated. Haplotypes in ATP8B1 demonstrated a significant difference between CC and control groups. There was also a trend towards significant association of V444A with CC. V444A has been strongly implicated in other cholestatic diseases. Given the biological plausibility of polymorphisms in ABCB11 and ATP8B1 as risk modifiers for CC, further study in a validation cohort is required.