induced significantly less naive T cell proliferation compared with MoDC matured in CM from uninvolved or inflamed liver tissue. Furthermore tumour-conditioned DCs generated significantly more CD4+CD25+FOXP3+ Tregs (p=0.01) and IL10producing T-cells (p=0.01). To determine the cell type responsible for this effect naive MoDCs were co-cultured with fibroblasts (-smooth muscle actin +vimentin+CD90+) isolated from either tumour cores or uninvolved liver. DCs conditioned by tumour fibroblasts developed a tolerogenic phenotype (MHCIIlowCD86low) and the ability to induce Tregs. Culturing MoDCs in tumour stroma CM had a similar effect implicating soluble factors. Part of this effect was IL-6-dependent because depletion of IL-6 from tumour-fibroblast CM abolished the ability to generate tolerogenic DC.

Conclusion Tumour associated fibroblasts in HCC contribute to an IL-6-rich TM that drives differentiation of tolerogenic DCs. These DCs generate immunosuppressive Tregs and IL-10 secreting T-cells which inhibit anti-tumour immunity. Inhibition of IL-6 or downstream STAT-3 signalling could prevent tumour-associated immunosuppression and hence be an important immunotherapeutic strategy in HCC.

P50 SYNTHETIC LETHALITY IN LIVER CANCER CELL LINES TREATED WITH INHIBITORS OF DNA DOUBLE-STRAND BREAK REPAIR

doi:10.1136/gut.2010.223362.76

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Introduction DNA double-strand breaks (DSBs) are the most cytotoxic lesions induced by ionising radiation (IR) and anticancer drugs, such as topoisomerase II poisons (eg, doxorubicin). The major DSB repair pathways are non-homologous end joining (NHEJ) and homologous recombination (HR), in which DNA-Dependent Protein Kinase (DNA-PK) and ataxia telangiectasia mutated (ATM) are key components. DNA-PK in particular is up-regulated in hepatocellular carcinoma, (GEO profiles) possibly contributing to resistance to cytotoxic therapies.

Aim To assess DNA-PK and ATM as therapeutic targets for chemo- and radio-sensitisation in hepatoma.

Method Basal protein levels and activities were determined by Western blot analysis in hepatoma cell lines. DNA-PK and ATM activity following doxorubicin stimulation was measured using antibodies specific to phosphorylated Ser-2056 DNA-PKcs and phosphorylated Ser-1981 ATM. DSB repair was measured by immunofluorescence detection of γ-H2AX foci. Cell survival was determined by clonogenic assay.

Results We demonstrated high basal levels of DNA-PK in three hepatoma cell lines (Huh7, Hep3B and HepG2), with DNA-PK activation induced by 0.25 μM doxorubicin. Despite similar DNA-PK activation, we observed differential sensitivity to doxorubicin (7%, 49% and 75% survival at 10 nM doxorubicin in Huh7, Hep3B and HepG2, respectively). HepG2 cells with the greatest resistance to doxorubicin displayed a 10-fold activation of ATM relative to the other cell lines. The DNA-PK inhibitor NU7441, increased doxorubicin and ionising radiation (IR) induced cytotoxicity in all cell lines (1.3 up to fourfold), correlating with a reduction in DSB repair measured by γ-H2AX foci. Importantly, in doxorubicin resistant HepG2 cells, while incubation with NU7441 or the ATM inhibitor (KU55933) alone, had minimal effects on cell survival (91% and 86%, respectively), their combination in the absence of a cytotoxic agent markedly inhibited cell survival (21%; p<0.001, ANOVA). The addition of 10 nM doxorubicin reduced survival to less than 5% of colonies.

Conclusion These findings support the clinical application of DNA-PK and ATM inhibitors as chemo- and radio-sensitisors in hepatoma patients. Furthermore, these data suggest that hepatoma cell survival is dependent on up-regulation of DSB repair, effected by either DNA-PK or ATM, and that inhibition of both induces synthetic lethality—preventing DSB repair by both NHEJ and HR. The therapeutic implication is that in combination, these agents could be used to specifically induce cancer cell death, with minimal toxicity to surrounding liver tissues.

P51 INADEQUATE COMPENSATION BY GLUTAMINE SYNTHETASE AND INCREASED GLUTAMINASE ACTIVITY CONTRIBUTES TO HYPERAMMONAEMIA IN CIRRHOSIS

doi:10.1136/gut.2010.223362.77

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Introduction In cirrhosis, the function of the urea cycle is compromised which leads to accumulation of ammonia. In this situation, ammonia metabolism is regulated by glutamine synthetase (GS) and glutaminase (GA) making them important therapeutic targets. The relative contributions of these enzymes in the different organs in regulating ammonia metabolism in cirrhosis are unclear.

Aim To study the protein expression and activity of glutamine synthetase (GS) and glutaminase (GA) enzymes in the different organs in a model of chronic liver disease (bile duct ligation: BDL).

Method Ten male Sprague—Dawley rats were studied (260.7±10.57) g: 4 sham operated, and 6 following bile duct ligation (BDL). We measured plasma levels for: ammonia and standard biochemical markers. Expression of GS and GA were determined by Western-blotting (described as % of sham expression) and activity by end point methods in liver, kidney, gut, muscle, lung and frontal cortex (brain).

Results Plasma ammonia was increased in BDL rats vs. Sham (45.97±14.72 vs 106.2±59.10) μmol/l). The most important organs for GS activity were the liver > lung = frontal cortex > muscle > kidney = gut. In cirrhosis, liver GS activity is reduced by 7 fold (62.61±8.29 SHAM vs 8.98±2.67* BDL). The most important organs for GA function in disease were: lung (0.70±1.4 SHAM vs 4.19±2.24* BDL) > kidney (1.24±0.09 SHAM vs 1.68±0.58* BDL) > gut (0.43±0.14 SHAM vs 1.14±0.51* BDL) (activities expressed as mIU/mg protein; *P<0.05).

Gut Liver Kidney Muscle Lung Frontal cortex Brain SHAM GS (0.78+0.67) (62.61+8.29) (0.87+1.24) (1.75+0.48) (2.98+4.26) (2.74+1.14) GA (0.43+0.14) (1.84+0.58) (1.24+0.09) (0.37+0.14) (0.70+1.4) (0.61+0.30) BDL GS (0.84+0.84) (8.98+2.67)* (0.86+0.78) (1.92+0.63) (2.15+3.14) (3.22+0.35) GA (1.14+0.51)* (0.52+0.16)* (1.68+0.58)* (0.38+0.11) (4.19+2.24)* (0.63+0.20).

Conclusion Inadequate compensation by GS and increased GA activity account for hyperammonemia observed in cirrhosis. For the first time, these data indicate the importance of the lung in regulating ammonia metabolism through GS and also GA, activities of both of which are increased in cirrhosis. In order to reduce ammonia levels in cirrhosis, it would be advantageous for novel drugs to target GS stimulation and Glutaminase inhibition simultaneously.

P52 NEUTROPHIL DYSFUNCTION: A POTENTIAL BIOMARKER OF POOR PROGNOSIS IN ACUTE LIVER FAILURE?

doi:10.1136/gut.2010.223362.78

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Introduction In acute liver failure (ALF) an exaggerated systemic inflammatory response can result in neutrophil activation with

subsequent tissue damage from release of proteolytic enzymes and reactive oxygen species, contributing to ongoing organ failure. Neutrophil function in ALF has not been previously interrogated.
Aim This ongoing longitudinal study aims to characterise neutrophil function in patients with ALF admitted to King's College Hospital.
Method Neutrophils were isolated from a cohort of age/sex matched patients with ALF (n=22), healthy volunteers (n=9) and septic controls (n=5). Serial samples were taken on admission and every 3–4 days following ITU admission until death/discharge. Phagocytosis was analysed by flow cytometry using FITC-labelled *E coli* and oxidative burst (OB) was determined by the percentage of CD16-Phycoerytherin labelled neutrophils producing reactive oxygen species at rest and after stimulation with opsonised *E coli*. Physiological variables, biochemistry, arterial ammonia and microbial culture results were collected prospectively.
Results Within the ALF cohort 14 patients fulfilled poor prognostic criteria, of whom 8 underwent successful liver transplantation (LT) and 6 died without LT, 8 survived with medical management. Aetiology of ALF was acetaminophen n=5; acute viral hepatitis n=4; seronegative liver failure n=10; drug/other n=3. APACHE II and SOFA scores on admission were higher in patients with ALF compared to septic controls 21 (17–25) vs 12 (9–15) (p=0.08) and 16 (15–17) vs 5 (1–6) (p=0.01) respectively. Impaired neutrophil phagocytosis ($p<0.01$) and increased spontaneous OB (p=0.05) was observed on admission in all patients with ALF compared to both control groups. Admission neutrophil phagocytic dysfunction was associated with higher CRP*, MELD*, INR* and SOFA scores* (*all $p<0.05$). Spontaneous OB deteriorates further on days 4–8 in poor prognostic groups compared to spontaneous survivors (p=0.038). This was accompanied by decline in OB in response to *E coli* which was also observed in septic controls (p=0.045). The defects in neutrophil function showed a trend towards improvement (phagocytosis, spontaneous and stimulated OB all p=ns) during the first 72 h following successful LT.
Conclusion In conclusion, in patients with ALF fulfilling "poor prognostic criteria" neutrophils demonstrate early impairment of phagocytosis, increased baseline OB but decreased OB in response to bacterial stimulation. These observed defects are likely to contribute to ongoing cellular/organ dysfunction and the increased susceptibility to nosocomial sepsis seen in ALF.

P53 THE CONTRASTING EFFECT OF OCTANOATE AND OLEATE ON PHOSPHATASE AND TENSIN HOMOLOGUE EXPRESSION IN IN VITRO MODEL OF STEATOSIS USING HEPG2/C3A CELLS

doi:10.1136/gut.2010.223362.79

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Introduction The tumour suppressor phosphatase and tensin homologue (PTEN) is mutated or deleted in several human cancers including hepatocellular carcinoma. PTEN-deficient mice demonstrated triglyceride accumulation, steatohepatitis, progressing to liver fibrosis and hepatocellular carcinoma. Similarly, reduced PTEN expression with free fatty acid (FFA) oleate has been shown to promote hepatic steatosis. In other cancer, mitochondrial respiration defect with enhanced glycolysis and NADH formation has been suggested to be a key event in PTEN downregulation.
Aim Our aims were to examine whether i) medium chain FFA octanoate altered PTEN expression ii) PTEN downregulation with FFA was associated with hepatic mitochondrial dysfunction.
Method Human hepatoblastoma cell line HepG2/C3A was pretreated for 3 days with oleate (0.25 mM) or octanoate (2 mM). PTEN expression was determined using quantitative real time PCR. Mitochondrial function was measured using BDTM oxygen biosensor in the presence of 2,4 dinitrophenol. Lactate and pyruvate concentrations were measured in the supernatant to determine glycolytic activity and NADH/NAD+ ratio. Intracellular lipid accumulation was confirmed with triglyceride concentrations. Experiments were done in triplicate to n=3. Results are expressed in mean±SEM. Differences between groups were analysed by one-way ANOVA.
Results We have previously demonstrated that oleate and octanoate pretreatment resulted in a similar intracellular triglyceride accumulation. In this study, we have found that despite similarities in triglyceride concentration, PTEN expression was lower in octanoate pretreated cells (octanoate 0.84±0.06, oleate 1.18±0.12, untreated 1.19±0.12 fold change from b-actin, p=0.04). However, octanoate pretreatment was not associated with impaired respiration (octanoate 0.24±0.01, oleate 0.20±0.02, untreated 0.28±0.01 AFU/gTP (gram of total protein)/min). Nevertheless, reduced PTEN expression with octanoate was associated with increased glycolysis (octanoate 315.2±42.91, oleate 100.9±14.09, untreated 145.3±8.83 μmol/gTP/hr, p=0.0001) with raised NADH/NAD ratio (octanoate 17.3±1.4, oleate 13.8±2.9 untreated 17.3±1.4; p=0.007).
Conclusion To our knowledge, the effect of octanoate on PTEN expression has not been previously shown. In contrast to the previous finding, our data demonstrate that octanoate, not oleate, downregulates PTEN expression. Differences in glycolysis hence redox potential may have influenced the disparity in PTEN expression between these FFA. Octanoate has recently been proposed to be beneficial in weight loss and diabetes. However, our findings suggest that it may not have a favourable effect on the progression of nonalcoholic fatty liver disease.

P54 VASCULAR ADHESION PROTEIN-1: A KEY PLAYER IN THE MODULATION OF HEPATIC FIBROSIS

doi:10.1136/gut.2010.223362.80

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Introduction Vascular adhesion protein-1 (VAP-1) is a membrane-associated amine oxidase present on sinusoidal endothelium that supports lymphocyte recruitment to the liver. We have recently detected increased expression of VAP-1 in cirrhotic livers, and reported that a circulating soluble form is elevated in patients with fibrotic liver disease leading us to propose that VAP-1 plays a broader role in hepatic fibrosis.
Aim To investigate the role of VAP-1 in the initiation, progression and resolution of hepatic fibrosis.
Method Human: The distribution and expression of VAP-1 was investigated using multicolour confocal microscopy of normal and diseased human liver. Hepatic stellate cells (aHSC) and activated liver myofibroblasts (aLMF) were isolated from human liver tissue. Cell proliferation was studied using CyQuant. Apoptosis was investigated using a caspase-3 flow cytometry approach and cell detachment assay. Cell spreading was evaluated using xCELLigence impedence measurements. Modified Boyden chambers were used to assess cell migration. Mouse: Liver fibrosis was induced by CCl4 administration in wild-type C57BL/6 mice, wild-type mice dosed with anti-"VAP"-1 antibody and VAP-1 null mice. The degree of fibrosis and inflammatory infiltrate was assessed during active fibrosis and subsequent resolution using immunohistochemistry and qRT-PCR.
Results VAP-1 was present on sinusoids and vascular endothelium in normal human liver but was markedly increased in expanded septa in fibrotic disease where it co-localised with markers of HSC and