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

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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

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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?

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