established human disease. In vitro when murine HPCs are co-cultured with myofibroblasts y-secretase inhibition significantly reduced expression of the Notch effectors HeyL ($p<0.001$ 3.133±0.32 vs 0.98±0.09) and Hey1 ($p<0.001$ 1.67±0.34 vs 0.08±0.01); associated with this reduction we found a significant reduction in expression of biliary genes HNF6 ($p<0.001$ 77.94±12.52 vs 14.97±4.57) and GGT ($p<0.01$ 6082±757.70 vs 3169±449.0). In vivo administration of DAPT during murine biliary regeneration demonstrates a significant reduction of cholangiocyte numbers ($p<0.05$ 957.7±114.2 vs 1787±266) and also a reduction in pro-biliary genes including HNF6 and HNF1β. NUMB, a negative regulator of Notch signalling is lost during murine biliary regeneration ($p<0.01$; 1.19±0.07 vs 0.10±0.02) as well as during PSC/PBC ($p<0.01$; 8748±3090 vs 1814±277). During regeneration of hepatocytes from HPCs in HCV and in murine models NUMB levels remain high and Notch signalling is suppressed. During murine hepatocyte regeneration NUMB is maintained via macrophage derived Wnt. In vitro activation of the Wnt pathway in HPCs resulted in a twofold induction of NUMB and 35-fold induction of HNF4, without significantly affecting HNF1β. We ablated macrophages using liposomal clodronate this resulted in a conversion of hepatocyte HPCs into biliary HPCs which formed luminal structures with membranous b-catenin, lost expression of Wnt pathway target Axin2, NUMB and HNF4 as well as inducing expression of Hes-1, HNF1β and HNF6.

Conclusion During regeneration of the adult liver, Notch signalling is required for biliary specification however is actively sequestered via NUMB during regeneration of hepatocytes. This hepatocyte phenotype is induced through signalling via the Wnt pathway, removal of which results in a biliary phenotype during hepatocyte regeneration. We also describe how this mechanism is conserved from mouse models to human disease, where during biliary diseases such as PSC and PBC the Notch pathway is activated, however is actively restricted during regeneration of in hepatocytes during HCV.

P45 THE EFFECT OF SUMO MODIFICATION ON HEPATIC DIFFERENTIATION FROM HUMAN EMBRYONIC STEM CELLS

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Introduction Protein post translational modifications (PTMs) play an important role in many cellular processes including; transcription, apoptosis, cell cycle regulation and cytoskeleton organisation. Sumoylation is a particular PTM which affects cellular activities such as chromatin organisation, protein localisation and cell cycle regulation. SUMO is a small ubiquitin like molecule. Typically it is covalently attached to lysine residues in the ψKxE consensus sequence (K- lysine, E - glutamic acid) although ~40% of SUMOylated proteins are modified on non-consensus sequences.

Aim SUMO modification has been shown to be important in hESC self-renewal where one of the master regulators, Oct4, is stabilised and its degradation is inhibited by SUMOylation (Zhang *et al*, 2007). We hypothesised that SUMO modification may not only regulate hESC self-renewal, but may also be required for efficient hESC differentiation. We therefore interrogated the role of SUMOylation in hESC differentiation to hepatic endoderm (HE). hESC were differentiated using our established and efficient model (Hay *et al*, 2008).

Method Cell lysates were collected at different time points throughout the differentiation and analysed by Western blotting for changes to the levels of key proteins involved in the conjugation and de conjugation of SUMO.

Results We demonstrate that peak levels of SUMOylation were detectable in hESC populations during cellular differentiation to definitive endoderm (DE). Following commitment to DE we observed a decrease in the level of SUMO modified proteins as the cells in culture developed a hepatic fate. This corresponded with an increase in SENP 1, a SUMO specific protease. We also detected reduced levels of HNF4α, a critical regulator of hepatic status and metabolic function, as SUMOylation decreased. As a result we investigated the role of SUMO modification in HNF4α metabolism and if this process was involved in modulating HNF4α's critical role in HE.

Conclusion In conclusion, SUMO modification and deconjugation at critical points during cellular differentiation may regulate protein stability enhancing transcriptional activity and/or modulating subcellular localisation. As a consequence this may improve HE differentiation, viability and maturity, which are essential to the generation of high fidelity human models in culture and maybe directly applicable to other stem cell populations for example iPSCs.

P46 VASCULAR ADHESION PROTEIN-1 PROMOTES INFLAMMATION AND FIBROGENESIS IN MURINE STEATOHEPATITIS

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Introduction VAP-1 is an adhesion molecule which promotes lymphocyte recruitment to the liver. It is released in a soluble form (sVAP-1) from adipose tissue and the hepatic vascular bed. sVAP-1 has insulin-like effects, can initiate and propagate oxidative stress and is implicated in vascular complications of the metabolic syndrome. Our group has discovered that VAP-1 is expressed and secreted by hepatic stellate cells and we have reported that serum sVAP-1levelsareelevated in NAFLD and predict fibrosis. These observations suggest that VAP-1 may have a role in mediating interactions between lymphocytes and stromal cells to promote inflammation induced fibrosis in NAFLD.

Aim To investigate a possible pathogenic role for VAP-1 in NAFLD by determining the effects of the inhibition or absence of VAP-1 in murine models of steatohepatitis.

Method 1. A high fat diet (HFD) was administered for 18 weeks in wild type (WT) C57Bl/6 mice (n=5) and VAP-1 null mice (n=5). 2. A methionine choline deficient (MCD) diet was administered for 6 weeks in WT mice (n=6), WT mice receiving an anti-VAP-1 antibody (n=6) and VAP-1 null mice (n=6).

Results In the HFD model VAP-1 null mice developed less steatosis on quantitative analysis of Oil Red O staining ($p<0.001$) and had fewer inflammatory foci ($p<0.05$) than WT mice. They were also protected against the onset of fibrosis with less collagen deposition ($p<0.001$) and lower levels of hepatic gene expression of SMA ($p<0.05$) and collagen 1, α 1 ($p<0.05$). In the MCD model VAP-1 null and antibody treated mice were equally protected from liver injury; showing less steatosis ($p<0.001$), fewer inflammatory foci ($p<0.001$), less collagen staining ($p<0.001$) and lower hepatic SMA ($p<0.001$) and collagen type 1, α 1 ($p<0.01$) mRNA expression than WT mice. Both models of steatohepatitis resulted in increased hepatic gene expression of VAP-1 when compared with age sex matched mice on normal diet.

Conclusion ElevatedsVAP-1 levels in NAFLD and increased hepatic VAP-1 expression in murine steatohepatitis suggest a role for VAP-1 in the pathogenesis of NAFLD. Inhibition and/or absence of VAP-1 are protective in two murine models of steatohepatitis implicating an important role for VAP-1 in hepatic fibrogenesis and suggesting it may be a potential therapeutic target in NAFLD.

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