P42 CHOLESTATIC PREGNANCY ALTERS CHOLESTEROL AND LIPID TRANSPORT TO THE FETAL CIRCULATION, AFFECTING LIPID METABOLISM OF THE FETUS

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Introduction Altered nutritional environment during intrauterine life may permanently affect tissue homeostasis of the fetus that can increase susceptibility of offspring to the development of disease in later life. Intrahepatic cholestasis of pregnancy (ICP) is a liver-specific disease of pregnancy that is characterised by increased bile acid (BA) levels in the maternal serum.
Aim We aimed to establish the effects of exposure of the fetus to increased BA levels as a result of maternal cholestasis in the mouse.
Method C57BL/6 mice were used for our studies (six mice/group). Cholestatic pregnancy was achieved with addition of 0.5% of cholic acid (CA) in the standard diet (ERD) 1 week before mating and up to day 18 of gestation when animals sacrificed. Biochemical measurements and gene and protein expression analyses took place in the maternal and fetal serum as well as maternal liver, fetal liver and placenta.
Results Consistent with a cholestatic profile, fetuses from CA-fed mothers were characterised by raised BA levels in the serum ($p<0.05$). Also, the hepatic BA receptor, Fxr, was activated as indicated by alterations in its target genes (reduced Cyp7a1, increased Shp and Bsep). As a result of cholestasis, fetal hepatic cholesterol and fatty acid biosynthesis were induced as proved by up-regulation of Srebp2, Srebp1c, Hmgcr and Fas ($p<0.05$). Moreover, fetal hepatic cholesterol and triglycerides were increased relative to the levels of fetuses of ERD-fed mothers. Comparison between the placentas of ERD- vs CA-fed mothers revealed that CA-fed placentas had increased expression levels of lipogenic-related genes such as Adrp, Ldlr and Acat-2 ($p<0.05$), accompanied by raised placental cholesterol and decreased ApoB ($p<0.05$).
Conclusion Cholestatic pregnancy leads to fetal cholestasis and increased hepatic cholesterol and fatty acid biosynthesis. The placental gene expression and biochemical profile imply that cholesterol accumulates in placenta instead of crossing into the fetal environment. Therefore, increased fetal hepatic cholesterol and fatty acid biosynthesis could be an adaptive mechanism of the fetus to fulfil their developmental nutritional demands. This may have adverse effects in later life of the offspring.

P43 ADHESION OF HUMAN BONE MARROW-DERIVED MESENCHYMAL STEM CELLS TO LIVER SINUSOIDAL ENDOTHELIUM IS BOTH CD29 AND CD44 DEPENDANT, WHILST INITIAL TETHERING OF MSCS TO ENDOTHELIUM INVOLVES CD29 BUT NOT CD44

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Introduction Whilst MSCs have been postulated to have a range of functions within the liver little is known about the factors regulating their migration, adhesion and localisation within liver tissue. Modulation of this process may have important therapeutic implications.
Aim To define the adhesion molecule profile on hMSCs and the molecular regulation of their interaction with liver sinusoidal endothelium.
Method Adhesion molecule expression of human bone marrow-derived mesenchymal stem cells (hBM-MSCs) was comprehensively profiled by flow cytometry before and after TNFα stimulation. Static adhesion assays were performed to assess binding to hBM-MSCs (±adhesion molecule blockade) to both extra-cellular matrix (ECM) proteins and sections of liver tissue. This was further assessed in flow assays over ECM and liver sinusoidal endothelial cells. MSC migration towards chemokine ligands was studied in Boyden assays.
Results hBM-MSCs expressed known markers (CD90/CD44/CD105) and demonstrated tri-lineage differentiation. Profiling of the adhesion molecules expressed by hBM-MSCs using flow cytometry found that they express high levels of integrin subunits (CD29 (β1), CD49c (α3), CD49d (α4)) and chemokine receptors CCR4 and CCR5. Furthermore, TNF stimulation increased expression of chemokine receptors including CCR4 and CCR5 by approximately 20%. In static binding asays, MSCs bound to immobilised ligands such as VCAM-1 and fibronectin, whose expression is increased in injured liver. In static liver tissue binding assays CD29 and CD44 blockade reduced binding to the liver sinusoids (30.9%±9 and 31%±7, respectively, both $p<0.05$), whilst only CD29 blockade significantly reduced binding to the parenchyma (36.2%±10 ($p<0.05$). Using a novel flow adhesion assay which re-creates venous blood flow in the liver sinusoids we observed tethering, but not rolling of hBM-MSCs when flowed over liver sinusoidal endothelium (LSEC). Interestingly we found that hBM-MSCs do not bind to LSEC under flow. Tethering of MSCs was ablated by blocking of CD29 on MSCs. Furthermore, using a modified stop-flow protocol we identified a role for CD29 and CD44 in firm adhesion of MSC to LSEC. hBM-MSCs migrated towards ligands for CCR4 and CXCR4 in Boyden chamber assays.
Conclusion Binding of hBM-MSCs to liver sinusoidal endothelium occurs via tethering and firm adhesion without prior rolling. Tethering is mediated by CD29, whilst firm adhesion of hBM-MSCs to LSEC involves CD29 and CD44 interactions. The ability of MSCs to migrate in response to CCR4 and CXCR4 ligands, along with studies showing upregulation of these ligands in injured liver tissue suggests that these receptors may be involved in controlling MSC migration to and/or within injured liver.

P44 A WNT-NUMB-NOTCH AXIS IS REQUIRED FOR LINEAGE SEPARATION OF HEPATIC PROGENITOR CELLS IN THE REGENERATING CELLS IN THE REGENERATING ADULT LIVER OF MOUSE AND HUMAN

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Introduction During chronic liver disease the liver can be regenerated from a population of endogenous hepatic progenitor cells (HPCs). These cells are known to be bipotent and can regenerate both hepatocytes and cholangiocytes, however the mechanisms by which these lineages are delineated from HPCs is currently unknown. Here we describe how the Notch and Wnt signalling pathways are differentially regulated and interact via NUMB to define cholangiocytes and hepatocytes from HPCs.
Results We have found that during biliary regeneration in primary sclerosing cholangitis and primary biliary cirrhosis, as well as mouse models of such, the Notch pathway is activated as demonstrated through nuclear Notch-1 in HPCs. This Notch mechanism is mediated through interaction of Jagged-1 supplied by a transient myofibroblast niche in mouse, or autonomous cell signalling in

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.