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