Basic science

P84 TOWARDS CELL BASED THERAPY FOR α_1 -ANTITRYPSIN DEFICIENCY THROUGH TARGETED BI-ALLELIC GENE CORRECTION IN HUMAN IPS CELLS

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Introduction Human induced pluripotent stem cells (hiPSCs) represent a unique opportunity for regenerative medicine since they offer the prospect of generating unlimited quantities of cells for autologous transplantation as a novel treatment for a broad range of disorders. We have previously developed disease specific human hepatocyte-like cells by reprogramming dermal fibroblasts taken from individuals with PiZ α_1 -antitrypsin deficiency. The resulting cells recapitulated the protein misfolding and intracellular polymer formation that characterise this disease (Rashid *et al*, *J Clin Invest* 2010;120:3127–36). However, use of iPS cells in treatment of individuals with PiZ α_1 -antitrypsin deficiency would also require correction of the underlying genetic abnormality in a manner fully compatible with clinical applications. The methods currently available, such as homologous recombination, lack the necessary efficiency and also leave residual sequences in the targeted genome.

Aim (1). To correct the genetic mutation responsible for PiZ α_1 -antitrypsin deficiency in human IPS cells in a clinically relevant way. (2). To investigate the potential of using corrected hiPS derived liver cells for cell based therapy by studying their in vivo function.

Method The genetic defect responsible for PiZ α_1 -antitrypsin deficiency (Glu342Lys) was targeted using a combination of engineered Zinc finger nucleases and a piggyBac vector in patient specific hiPSCs. Corrected cells were differentiated to hepatocytes using a previously optimised chemically defined in vitro platform. Assessment of phenotypic correction was then made in vitro as well as in vivo.

Results We demonstrate here for the first time an efficient gene editing technique capable of restoring normal structure, function and secretion of α_1 -antitrypsin in subsequently derived liver cells without leaving residual exogenous sequences in the targeted hiPSC genome. Moreover injection of corrected hiPS derived liver cells into a mouse model of liver injury confirmed their potential for functional capacity in vivo. Importantly none of the transplanted mice showed evidence of tumour formation.

Conclusion Our results provide proof of principle for the potential of combining hiPSCs with gene therapy to generate cells for autologous cell based treatment of α_1 -antitrypsin deficiency in humans.

P85 HUMAN MESENCHYMAL STEM CELLS BIND PREFERENTIALLY TO INJURED LIVER IN A β_1 -INTEGRIN AND CD44 DEPENDENT MANNER

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Introduction Human bone-marrow mesenchymal stem cells (hMSCs) are multipotent cells which have been considered in liver repair due to their immunomodulatory properties. However, they have also been implicated in liver fibrogenesis. These differing actions may reflect the existence of different functional sub-sets of hMSCs or differing environmental cues. Understanding the mechanisms governing the entry and retention of hMSCs into liver would allow this to be modified for clinical benefit in disease settings.

Aim To determine the mechanisms underpinning the adhesion and engraftment of human MSCs into injured liver.

Method Flow cytometric analysis of hMSCs was performed to determine the expression of adhesion molecules corresponding to up-regulated ligands in injured liver. Adhesion of hMSCs to normal and injured liver tissue, human hepatic sinusoidal endothelium (HSEC) and extracellular matrices (VCAM-1, fibronectin and hyaluronan) was undertaken in vitro using static and physiologically relevant modified-flow assays. Fluorescently-labelled hMSCs were infused via the portal vein into mice injured with chronic CCl₄ to determine engraftment. To define the molecular mechanisms underpinning hMSC interactions neutralising antibodies were utilised in these assays.

Results hMSCs expressed high levels of β_1 -integrin and CD44. Adhesion of hMSCs was greatest on diseased human liver compared to normal liver. Neutralising antibodies against β_1 -integrin, CD44 and VCAM-1 reduced binding of hMSCs to diseased liver by 34%, 35% and 40% respectively ($p < 0.05$). hMSCs were seen to fast-roll at 500 μ m/s, firmly adhere and transmigrate across HSEC. Rolling was completely abolished by β_1 -integrin blockade on both HSEC and VCAM-1. Firm adhesion to HSEC was reduced by blockade of β_1 -integrin (55%, $p = 0.02$) and CD44 (51%, $p = 0.04$). To determine which ligands on HSEC mediate hMSC binding we studied the ligands in isolation. HSEC express VCAM-1, fibronectin and hyaluronan, all of which supported firm adhesion of hMSCs under modified flow. β_1 -integrin blockade reduced adhesion to VCAM-1 (66%, $p = 0.001$) and Fibronectin (29%, $p = 0.007$), while CD44 blockade reduced adhesion to fibronectin (18%, $p = 0.02$) and hyaluronan (43%, $p = 0.04$). Neutralising antibodies to the β_1 -integrin subunit and CD44 reduced hepatic engraftment of hMSCs in chronically injured mice, confirming the data obtained in vitro.

Conclusion hMSCs bind preferentially to injured liver and undergo rolling and firm adhesion, which is differentially regulated. β_1 -integrin/VCAM-1 interactions regulate rolling while β_1 -integrin/CD44 interactions with fibronectin and hyaluronan regulate adhesion.

P86 5 α -REDUCTASE-1 KNOCKOUT PROMOTES STEATOSIS BUT PROTECTS AGAINST HEPATOCARCINOGENESIS IN A MURINE MODEL OF NAFLD

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Introduction Glucocorticoids have been implicated in the pathogenesis of NAFLD, but as most patients with NAFLD have normal circulating cortisol levels it has been suggested that it is hepatic levels which are important. 5 α -reductase (5 α R) clears cortisol, as well as converting testosterone to the more active androgen dihydrotestosterone. Its role in the pathogenesis of NAFLD has yet to be determined.

Aim To determine the role of 5 α R in the pathogenesis of NAFLD.

Method Human liver samples from patients with NAFLD (scored by Kleiner classification) and normal donor tissue were used for immunohistochemical/real-time PCR analysis. Wild type (WT) ($n = 20$) and 5 α R1 knockout (KO) mice ($n = 20$) were fed either normal chow or American lifestyle induced obesity syndrome (ALIOS) diet for 6 or 12 months. ALIOS diet is high in trans-fats and high fructose corn syrup. Glucose tolerance, liver and body weights were recorded and liver histology graded (Kleiner). Hepatic triglycerides and mRNA markers of lipid metabolism were quantified. PanCK immunohistochemistry (oval cell response) was quantified by image analysis.

Results 5 α R1 expression in human livers was parenchymal and greater in non-alcoholic steatohepatitis (NASH) livers compared to normal liver and was associated with greater lobular inflammation. 5 α R1 gene expression was not affected by NASH severity. ALIOS induced steatohepatitis (6 m) and significant fibrosis (F3 at 12 m) in WT and 5 α R1 KO mice. Compared with WT mice, 5 α R1 KO mice fed the ALIOS diet demonstrated significantly greater liver weight, steatosis score (median 3 vs 1), and hepatic triglyceride accumulation by 6 months (93.1 vs 62.4 nmol/mg $p=0.002$). mRNA expression of CPT1 α , a key enzyme in hepatic fatty acid β oxidation, was reduced in 5 α R1 $^{-/-}$ mice. There was a trend ($p=0.1$) towards worse inflammation (Kleiner lobular inflammation/qPCR for hepatic TNF α) at 6 months in 5 α R1 KO mice, but no difference in inflammation or fibrosis at 12 months despite the presence of greater hepatic steatosis. The number of panCK-positive cells observed in WT mice fed ALIOS diet increased significantly with longer duration (0 m 0.15%, 6 m 0.44%, 12 m 0.94%, $p=0.028$) and at 12 months was significantly greater than mice fed normal chow (0.94% vs 0.18% $p=0.03$). Despite the absence of cirrhosis 5/10 WT mice developed dysplasia/hepatocellular carcinoma after ALIOS for 12 months vs 0/5 5 α R1 KO mice. 5 α R1 KO mice had lower numbers of panCK-positive cells (0.62% vs 0.94%) compared to its WT.

Conclusion ALIOS diet induces the entire spectrum of NAFLD from simple steatosis to advanced NASH with fibrosis and HCC, with a mounting oval cell response to increasing duration of diet. 5 α R1 KO promotes hepatic steatosis in the absence of worsening fibrosis, attenuates the oval cell response and exerts a protective effect on hepatocarcinogenesis thereby demonstrating the role of 5 α R in NAFLD pathogenesis.

P87 VASCULAR ADHESION PROTEIN-1 PROMOTES INCREASES IN LIVER INFILTRATING CD4 $^{+}$ T CELLS AND NKT CELLS AND INDUCTION OF FIBROGENESIS IN STEATOHEPATITIS

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Introduction Vascular Adhesion Protein-1 (VAP-1) is an adhesion molecule and membrane-bound amine oxidase. Our group has previously demonstrated that VAP-1 is able to support lymphocyte recruitment across human hepatic sinusoidal endothelium in vitro. Soluble VAP-1 (sVAP-1) is released into the circulation from adipose tissue and the hepatic vascular bed, has insulin like effects and is capable of inducing and propagating oxidative stress.

Aim To investigate the role of VAP-1 in the progression of steatosis to steatohepatitis and cirrhosis.

Method Human hepatic VAP-1 expression was determined using immunofluorescent labelling and multicolour confocal microscopy. Serum sVAP-1 levels were measured by ELISA in 144 patients with histologically graded NAFLD and 74 controls matched for age and metabolic phenotype. Two murine models of steatohepatitis were studied; (1) a methionine choline deficient diet was administered for 6 weeks in wild-type (WT) mice, WT mice treated with anti-VAP-1 antibody, and VAP-1 null mice ($n=6$ per group), 2) A western lifestyle model incorporating high trans-fat diet and fructose supplemented drinking water was administered to WT and VAP-1 null mice for 6, 9 and 12 months ($n=10$ per group). Control animals remained on normal chow. Liver infiltrating lymphocytes were identified and quantified using flow cytometry. Fibrosis was assessed by immunohistochemistry and qRT-PCR for α SMA and collagen I.

Results (1) Human studies: We report increased hepatic expression of VAP-1 in NAFLD associated with elevated serum levels of sVAP-1

compared with controls (946 ± 468 ng/ml vs 265 ± 78 ng/ml, $p<0.0001$). Multiple regression modelling reveals sVAP-1 to be the best predictor of histological fibrosis in our cohort. We detected strong VAP-1 expression in fibrotic septa co-localised with activated liver myofibroblasts (aLMF). In vitro human aLMF expressed and released sVAP-1 which promoted lymphocyte migration through a novel H₂O₂-mediated enzyme-dependent mechanism. (2) Experimental steatohepatitis: VAP-1 null and/or antibody treated mice developed fewer inflammatory foci and delayed onset of fibrosis in two murine models of NASH. In both models there was a specific failure to expand the intrahepatic CD4 $^{+}$ and NKT cell populations in VAP-1 null mice compared with 2 to 3-fold increases in WT mice. Three WT animals developed hepatocellular carcinoma by 12 months in the western lifestyle model but no tumours were found in VAP-1 null mice.

Conclusion Our results implicate an important role for VAP-1 in steatohepatitis, in both humans and mice. The ability to target VAP-1 with antibodies or small molecule inhibitors makes it an attractive therapeutic target.

P88 VASCULAR ADHESION PROTEIN-1 (VAP-1) MODULATES GLUCOSE AND LIPID UPTAKE IN NON-ALCOHOLIC FATTY LIVER DISEASE (NAFLD)

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Introduction NAFLD is characterised by steatosis, chronic inflammation and fibrosis. The underlying mechanisms include insulin resistance, increased free fatty acid flux from de-novo lipogenesis and decreased lipid oxidation. Vascular Adhesion Protein-1 (VAP-1), is an adhesion molecule with semicarbazide-sensitive amine oxidase activity (SSAO), which is also expressed as a soluble protein in serum (sVAP-1) and elevated in inflammatory liver diseases such as NAFLD. VAP-1 has been shown to modulate glucose and lipid uptake in muscle and adipose tissue and thus we investigated whether it may contribute to glucose and lipid homeostasis in human liver tissue.

Method We have used precision cut liver slices (PCLS) from normal and diseased human liver specimens and cultured human sinusoidal endothelium (HSEC) and hepatocyte cells in combination with VAP-1 substrates (200 μ M methylamine or benzylamine) and inhibitors (400 μ M bromoethylamine) to perform standard ex vivo radiolabelled glucose uptake and fatty acid uptake assays using oil red O quantification following exposure of cells to Oleic and Palmitic acid (PA). Immunohistochemical staining and qPCR were performed using standard techniques and for confirmatory experiments HSEC were transfected with enzymatically active/inactive VAP1.

Results QPCR confirmed upregulation of VAP-1 mRNA ($\Delta\Delta CT = 1.144$ $p=0.03$) in NASH vs normal liver and also changes in FABP1, -4, -5, FATP3, -4 ($p\leq 0.05$ for all) and GLUT-1, 2, 3, 5, 8, 9 and 12 in NAFLD compared to normal individuals. Results were confirmed using immunohistochemical staining. Exposure of human PCLS to sVAP-1 and methylamine typically resulted in a 38–54% increase in PA uptake ($p\leq 0.01$ for all) and a 20% increase in hepatocyte glucose uptake in vitro which could be inhibited using bromoethylamine. Transduction of HSEC with enzymatically active VAP-1 also increased glucose uptake which was prevented in the absence of enzyme activity. Interestingly methylamine treatment of human liver resulted in decreased expression of mRNA for glucose transporters and an increase in some lipid transporters including FABP6, FATP and LRP8, and H₂O₂ produced by SSAO activity increase lipid uptake by hepatic cells.