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 α1-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 α1-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 α1-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 α1-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 α1-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 α1-antitrypsin deficiency in humans.

P85 HUMAN MESENCHYMAL STEM CELLS BIND PREFERENTIALLY TO INJURED LIVER IN A β1-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 CCl_4 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 μm/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 5α-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. 5α-reductase (5αR) 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 5αR 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 5αR1 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.