

PMO-133 A NATURALLY OCCURRING MODEL OF LIVER REGENERATION? CHANGES IN MRNA EXPRESSION OF MARKERS OF HEPATIC REGENERATION IN LIVER TISSUE FROM DOGS WITH CONGENITAL PORTOSYSTEMIC SHUNTS FOLLOWING PARTIAL ATTENUATION

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Introduction There has been increasing interest in the possibility of using spontaneous canine hepatic disease as a model for those in human beings.¹ Dogs with congenital portosystemic shunts (CPSS) have hypoplasia of the liver and intrahepatic portal veins. While the condition is extremely rare in people, it is more common in dogs. Surgical CPSS attenuation results in liver growth and development of the intrahepatic portal vasculature, associated with clinical improvement.² The precise mechanism of this hepatic response is unknown; we hypothesised that it is due to hepatic regeneration and angiogenesis. The study aimed to measure the mRNA expression of growth factors and receptors involved in liver regeneration and angiogenesis in liver biopsies from dogs with CPSS before and after partial attenuation.

Methods Dogs treated for CPSS were prospectively recruited to the study and liver biopsy samples were collected and placed in RNAlater. The expression of nine genes related to liver regeneration and angiogenesis were evaluated using quantitative polymerase chain reaction (qPCR). Differences in gene expression were assessed using independent or paired T tests. Significance was set at the 5% level (p=0.05).

Results Liver biopsies were collected from 49 CPSS dogs to seven control dogs. 24 dogs tolerated complete attenuation of their CPSS and 25 tolerated partial attenuation. A second surgery was performed in all partial attenuation dogs to achieve complete attenuation and a follow-up biopsy was taken. HGF mRNA expression was significantly decreased in CPSS dogs compared with controls. There were significant increases in mRNA expression of HGF, MAT2 α and VEGFR2 following partial CPSS attenuation. In addition, dogs that could tolerate complete attenuation had significantly greater MAT2 α , VEGFR2 and TGF β R2 mRNA expression.

Conclusion The results of this study indicate that the liver regeneration and angiogenesis are involved in the hepatic response to surgery in dogs with CPSS. This suggests that canine CPSS could be a useful, naturally occurring model of liver regeneration.

Competing interests None declared.

REFERENCES

1. Kruitwagen HS, Arends B, Spee B, et al. Recombinant hepatocyte growth factor treatment in a canine model of congenital liver hypoplasia. *Liver Int* 2011;**31**:940–9.
2. Lee KC, Lipscomb VJ, Lamb CR, et al. Association of portovenographic findings with outcome in dogs receiving surgical treatment for single congenital portosystemic shunts: 45 cases (2000–2004). *J Am Vet Med Assoc* 2006;**229**:1122–9.

PMO-134 BASAL CELL ADHESION MOLECULE AND β 1-INTEGRINS REGULATE THE ADHESION OF ES CELL-DERIVED HEPATOCYTE-LIKE CELLS TO EXTRACELLULAR MATRIX AND HEPATIC SINUSOIDAL CELLS

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Introduction Cellular transplantation is an alternative to liver transplantation, however provision of primary human hepatocytes

is limited. Human embryonic stem cell-derived hepatocyte-like cells (ES-HLCs) represent a renewable source of functional hepatocytes. However, engraftment levels of these cells in the liver is low. The mechanisms regulating interactions between transplanted hepatocytes and the host liver remain unclear. Elucidation of these mechanisms may provide a means to enhance recruitment and engraftment in vivo.

Methods ES-HLCs were generated using our previously published protocol.¹ Functionality of cells was demonstrated biochemically and by transplantation into immunocompromised fumaryl acetoacetate hydrolase knock-out (FAH^{-/-}) mice. Microarray analysis of adhesion molecule expression was undertaken and compared with primary human hepatocytes. Adhesion molecule expression of ES-HLCs was assessed by flow cytometry. Adhesion of ES-HLCs to extracellular matrices and human hepatic sinusoidal endothelium (HSEC) was quantified using static and physiologically relevant flow assays, respectively. To define the mechanisms underpinning ES-HLCs interactions adhesion molecule neutralising antibodies and a recombinant human BCAM protein (BCAM-fc) were utilised.

Results ES-HLCs displayed markers of functional hepatocytes and resulted in prolonged survival in FAH^{-/-} mice after intra-splenic transplantation. A range of genes for novel adhesion molecules were identified on ES-HLCs including BCAM. ES-HLCs expressed high levels of β 1-integrin (84.3 \pm 2.3%), as well as high levels of BCAM (84.3 \pm 2%). In static adhesion assays, ES-HLCs bound preferentially to laminin, fibronectin and osteopontin. Binding to fibronectin and osteopontin was reduced when β 1-integrin was blocked (28.6% \pm 6.5 p=0.022 and 34% \pm 6.5 p=0.008, respectively). Furthermore, adhesion to laminin was reduced by 48.6 \pm 3% (p=0.03) when cells were treated with an anti-BCAM blocking antibody or 48 \pm 5.1% (p<0.0001) when laminin-binding sites were blocked by BCAM-fc. Blockade of β 1-integrins on ES-HLCs or BCAM binding sites on HSEC led to significant (15% \pm 3 and 24% \pm 5, p=0.029 and 0.035, respectively) decreases in adhesion of ES-HLCs to HSEC during flow assays.

Conclusion Using microarrays we have identified novel adhesion molecules on ES-HLCs such as BCAM along with more established adhesion molecules such as β 1-integrins. These molecules critically regulate the adhesion of ES-HLCs to specific ECM molecules and HSEC in physiologically relevant flow assays. BCAM and β 1-integrins are thus potential targets to manipulate to improve the engraftment of transplanted ES-HLCs.

Competing interests None declared.

REFERENCE

1. Hay, et al. PNAS 2008;**105**:12301–6.

PMO-135 ZINC FINGER E-BOX BINDING HOMEBOX 1 (ZEB1) INDUCES EPITHELIAL TO MESENCHYMAL TRANSITION (EMT) AND PROMOTES TUMOUR PROGRESSION IN HEPATOCELLULAR CARCINOMA (HCC)

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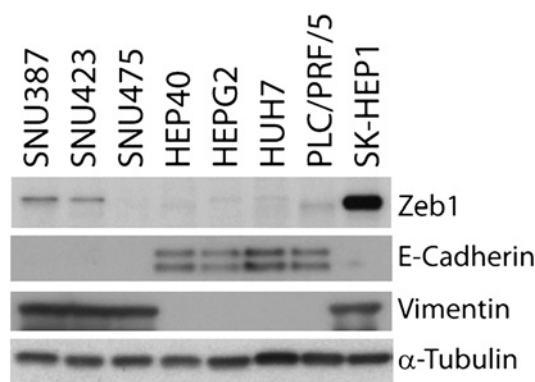
Introduction HCC is the leading cause of cancer related mortality world wide. Emerging evidence suggests aberrant activation of an embryological trans-differentiation programme termed epithelial-mesenchymal transition (EMT) is critical in promoting metastasis in different carcinomas. We analysed the expression of ZEB1, a key transcription factor implicated in EMT, by immunohistochemistry (IHC) and western blotting in HCC. Additionally, we performed migration assays to analyse the consequences of ZEB1 expression in Huh7 and HepG2 cell lines.

Methods We performed western blotting for ZEB1, E-cadherin, vimentin and α -tubulin to identify the epithelial-mesenchymal status of eight primary HCC cell lines. IHC was undertaken on paraffin sections from 40 patients who underwent resections for primary HCC between May 1997 and November 2010 and scored by two independent pathologists. Clinicopathological data were collated retrospectively and patient survival calculated using the Kaplan–Meier method. We transfected ZEB1 into Huh7 and HepG2 cell lines by electroporation and assessed EMT related changes in cell motility using Boyden chambers (pore size: 8 μ m) and serum as chemo-attractant.

Results Western blotting of proteins from eight HCC cell lines demonstrated reciprocal expression of ZEB1 and E-cadherin, suggesting EMT promotes a migratory phenotype in HCC. ZEB1 also significantly increased cell motility as a threefold increase in cell migration was observed after ZEB1 transfection into Huh7 cells (23 ± 4 vs 79 ± 5). ZEB1 positivity was detected in 11/40 specimens analysed by IHC. Statistical analysis highlighted ZEB1 as an independent prognostic marker favouring a significant reduction in cancer specific (41 vs 16 months, p

Conclusion Our results suggest that ZEB1 induced EMT promotes tumour progression and metastasis in HCC, and that over-expression of ZEB1 may represent an independent prognostic biomarker in patients with HCC.

Competing interests None declared.



Abstract PMO-135 Figure 1

PMO-136 DEFECTIVE INHIBITORY MOLECULES EXPRESSION MAY CONTRIBUTE TO BREAKDOWN OF TOLERANCE CHARACTERISTIC OF AUTOIMMUNE LIVER DISEASE

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Introduction Autoimmune hepatitis (AIH) is a severe hepatopathy often progressing to end-stage liver disease. Evidence implicates the involvement of both CD4 and CD8 T cell responses in its pathogenesis. There are a number of different inhibitory molecules expressed by T cells that can attenuate T cell receptor signalling. These include cytotoxic T lymphocyte antigen-4 (CTLA-4), programmed death-1 (PD-1), and the recently described T cell immunoglobulin and mucin domain-3 (Tim-3). Whether a disturbed expression of these inhibitory molecules can result in an increased susceptibility to autoimmune liver disease is unknown.

Aims to evaluate the expression of CTLA-4, PD-1, and Tim-3 by CD4 and CD8 T cells in patients with autoimmune hepatitis.

Methods 12 ANA/SMA+ AIH patients (6 females, median age: 14 years) and 6 healthy subjects (HS, four females, median age:

26.4 years) were studied. Phenotype of CD4 and CD8 T cells was determined by flow cytometry using monoclonal antibodies against CD4, CD8, PD-1 and Tim-3. Expression of CTLA-4 was determined by intracellular staining.

Results The frequency of Tim-3^{pos} and PD-1^{pos} cells within CD4 and CD8 T cells was lower in AIH (CD4^{pos}Tim-3^{pos}: 1.6 ± 0.3 ; CD4^{pos}PD-1^{pos}: 4.8 ± 0.5 ; CD8^{pos}Tim-3^{pos}: 9.6 ± 1.6 ; CD8^{pos}PD-1^{pos}: 6.7 ± 0.7) than in HS (CD4^{pos}Tim-3^{pos}: 6.2 ± 0.8 , PposPD-1^{pos}: 8.1 ± 1.9 , $P=0.04$; CD8^{pos}Tim-3^{pos}: 15.8 ± 1.8 , $P=0.04$; CD8^{pos}PD-1^{pos}: 12.6 ± 1.4 , 6.7 ± 0.7 , Ppos cells between the two groups of subjects. While in health dually Tim-3 and PD-1 positive populations are recognisable (CD4^{pos}Tim-3^{pos}PD-1^{pos}: 0.7 ± 0.1 ; CD8^{pos}Tim-3^{pos}PD-1^{pos}: 1.7 ± 0.4), they are reduced in AIH (CD4^{pos}Tim-3^{pos}PD-1^{pos}: 0.4 ± 0.1 , $p=0.007$; CD8^{pos}Tim-3^{pos}PD-1^{pos}: 0.9 ± 0.3 , $p=0.03$).

Conclusion AIH patients have fewer PD-1 and Tim3 positive cells within both CD4 and CD8 T cells. Defective expression of these negative immune-regulatory molecules may contribute to breakdown of tolerance, possibly accounting for the initiation and/or perpetuation of the autoimmune liver attack.

Competing interests None declared.

PMO-137 PHENOTYPIC AND FUNCTIONAL SIGNATURE OF CD4^{pos}CD25^{high}CD127^{low} REGULATORY T-CELLS IN AUTOIMMUNE HEPATITIS

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Introduction In autoimmune hepatitis (AIH) CD4^{pos}CD25^{high} regulatory T-cells (T-regs), a subset central to immune-tolerance, are numerically defective and impaired in their ability to control effector cell function. At variance with CD4 effectors, T-regs, classically known as CD25^{high} and FOXP3^{pos}, express low levels of the activation marker CD127. The aim of the current study was to provide a phenotypic and functional profile of CD4^{pos}CD25^{high}CD127^{low}T-regs (CD127^{low}T-regs) in AIH and to explore to what extent absence or low levels of CD127 impact on T-reg ability to suppress.

Methods 20 ANA/SMA+ AIH patients and 12 healthy subjects (HS) were studied. T-reg phenotype was determined by flow cytometry using antibodies to CD4, CD25, CD127, CTLA-4 and Galectin-9, a molecule linked to T-reg ability to suppress. T-reg transcription factor and cytokine profile were assessed by intracellular staining. CD127^{low}T-reg ability to suppress was evaluated in a proliferation assay following co-culture with CD25^{neg} target cells.

Results In AIH CD4^{pos}CD25^{high} cells contained fewer CD127^{low} cells than in HS. Compared to conventional CD4^{pos}CD25^{high} (cT-regs), CD127^{low}T-regs from both AIH and HS had a) higher numbers of FOXP3^{pos}, CTLA-4^{pos}, Galectin-9^{pos} and IL-10^{pos} cells; b) lower numbers of T-bet^{pos}, RORC^{pos}, IFN γ ^{pos} and IL-17^{pos} cells; and c) similar numbers of TGF- β ^{pos} cells. In AIH, CD127^{low}T-regs contained fewer FOXP3^{pos}, CTLA-4^{pos}, Galectin-9^{pos}, IL-10^{pos} and TGF- β ^{pos} cells and higher frequencies of T-bet^{pos}, RORC^{pos}, IFN γ ^{pos} and IL-17^{pos} cells than in HS. CD127^{low}T-regs inhibited CD25^{neg} cell proliferation more effectively than cT-regs, though less markedly in AIH than in HS. In AIH, treatment with anti-IFN γ and anti-IL-17 neutralising antibodies ameliorated the suppressive ability of cT-regs, while leaving unchanged that of CD127^{low}T-regs; exposure to anti-IL-10 neutralising antibodies reduced cT-reg suppression in HS, but not in AIH.

Conclusion CD127^{low} T-regs bear the phenotypic and functional signature of "true T-regs". Low numbers and reduced suppressive function of CD127^{low}T-regs in AIH may contribute to breakdown of