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, MAT2α and VEGFR2 following partial CPSS attenuation. In addition, dogs that could tolerate complete attenuation had significantly greater MAT2α, VEGFR2 and TGFβ2 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

BASAL CELL ADHESION MOLECULE AND B1-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 B1-integrin (34.5±2.3%), as well as high levels of BCAM (34.5±2.2%). In static adherence assays, ES-HLCs bound preferentially to laminin, fibronectin and osteopontin. Binding to fibronectin and osteopontin was reduced when B1-integrin was blocked (28.6±6.5 p=0.022 and 34.5±6.5 p=0.008, respectively). Furthermore, adhesion to laminin was reduced by 48.6±3% (p=0.03) when cells were treated with an anti-BCAM blocking antibody or 48.5±1% (p<0.0001) when laminin-binding sites were blocked by BCAM-fc. Blockade of B1-integrins on ES-HLCs or BCAM binding sites on HSEC led to significant (15.3±3 and 24.5±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 B1-integrins. These molecules critically regulate the adhesion of ES-HLCs to specific ECM molecules and HSEC in physiologically relevant flow assays. BCAM and B1-integrins are thus potential targets to manipulate to improve the engraftment of transplanted ES-HLCs.

Competing interests None declared.

REFERENCE

ZINC FINGER E-BOX BINDING HOMEODEX 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 worldwide. 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.

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1. Sreekumar, a Mirmiezami, H Wilson, J Primrose, G Thomas. Cancer Sciences Division, Southampton General Hospital, Southampton, UK

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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
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 μm) 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 = 0.03).

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.

PMO-137 PHENOTYPIC AND FUNCTIONAL SIGNATURE OF CD4POSCD25HIGHCD127 LOW REGULATORY T-CELLS IN AUTOIMMUNE HEPATITIS

Introduction In autoimmune hepatitis (AIH) CD4posCD25high 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 CD25hi and FOXP3pos, express low levels of the activation marker CD127. The aim of the current study was to provide a phenotypic and functional profile of CD4posCD25hiCD127lowT-regs (CD127lowT-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. CD127lowT-reg ability to suppress was evaluated in a proliferation assay following co-culture with CD25pos target cells.

Results In AIH CD4posCD25hiCD127low cells contained fewer CD127lowT-regs than in HS. Compared to conventional CD4posCD25hi (cT-regs), CD127lowT-regs from both AIH and HS had a) higher numbers of FOXP3pos, CTLA-4pos, Galectin-9pos and IL-10pos cells; b) lower numbers of T-betpos, RORCpos, IFNγpos and IL-17pos cells; and c) similar numbers of TGF-bpos cells. In AIH, CD127lowT-regs contained fewer FOXP3pos, CTLA-4pos, Galectin-9pos, IL-10pos and TGF-bpos cells and higher frequencies of T-betpos, RORCpos, IFNγpos and IL-17pos cells than in HS. CD127lowT-regs inhibited CD25pos cell proliferation more effectively than cT-regs, though less markedly in AIH than in HS. In AIH, treatment with anti-IFNγ and anti-IL-17 neutralising antibodies ameliorated the suppressive ability of cT-regs and neutralising antibodies in CD127lowT-regs, though less markedly in AIH than in HS. In AIH, treatment with anti-IFNγ and anti-IL-17 neutralising antibodies did not reduce CD127lowT-reg ability to suppress.

Conclusion CD127low T-regs bear the phenotypic and functional signature of “true T-regs”. Low numbers and reduced suppressive function of CD127lowT-regs in AIH may contribute to breakdown of
PMO-135 Zinc finger e-box binding homeobox 1 (ZEB1) induces epithelial to mesenchymal transition (EMT) and promotes tumour progression in hepatocellular carcinoma (HCC)

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