

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

doi:10.1136/gutjnl-2012-302514b.133

¹M S Tivers,* ¹V Lipscomb, ²K Smith, ³C Wheeler-Jones, ⁴A House. ¹Department of Veterinary Clinical Sciences, Royal Veterinary College, Hatfield, UK; ²Department of Pathology and Infectious Diseases, Royal Veterinary College, Hatfield, UK; ³Department of Veterinary Basic Sciences, Royal Veterinary College, Hatfield, UK; ⁴Veterinary Referral Hospital, Hallam, Australia

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

doi:10.1136/gutjnl-2012-302514b.134

¹N J Davies,* ¹H S Dawes, ¹D W Blakeway, ¹P F Lalor, ¹D H Adams, ²D C Hay, ¹P N Newsome. ¹NIHR Biomedical Unit and Centre for Liver Research, University of Birmingham, Birmingham, UK; ²MRC Centre for Regenerative Medicine, University of Edinburgh, Edinburgh, UK

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)

doi:10.1136/gutjnl-2012-302514b.135

R Sreekumar,* A Mirnezami, H Wilson, J Primrose, G Thomas. *Cancer Sciences Division, Southampton General Hospital, Southampton, UK*

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.