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 threelfold 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<0.05).

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, 4 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; P<0.04; CD8 pos PD-1 pos: 12.4±1.4 6.7±0.7, P<0.05). 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<0.05).

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 CD4posCD25highGHC127 LOW REGULATORY T-CELLS IN AUTOIMMUNE HEPATITIS

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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 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 C-D127 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 pos target cells.

Results

In AIH CD4posCD25high cells contained fewer CD127 low cells than in HS. Compared to conventional CD4posCD25 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, IFNγ pos and IL-17 pos cells; and c) similar numbers of TGF-b pos cells. In AIH, CD127 low T-regs contained fewer FOXP3 pos, CTLA-4 pos, Galectin-9 pos, IL-10 pos and TGF-b pos cells and higher frequencies of T-bet pos, RORC pos, IFNγ pos and IL-17 pos cells than in HS. CD127 low T-regs inhibited CD25 pos 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, 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