TREM-2 defends the liver against Hepatocellular Carcinoma through multifactorial protective mechanisms

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Poster number: P84

Conflict of interests: No

INTRODUCTION

Hepatocellular carcinoma (HCC) represents the 6th most common cancer world-wide and the 3rd leading cause of cancer-related mortality. 80% of the HCCs arise in cirrhotic livers, highlighting the strong association of this tumour with underlying chronic liver diseases driven by inflammation and regeneration. We have previously shown that the triggering receptor expressed on myeloid cells 2 (TREM-2) protects the liver from hepatotoxic injury, via its negative regulation on toll-like receptor (TLR)-derived signalling in non-parenchymal liver cells. However, its role in liver cancer is still far from clear.

Here, the role of TREM-2 in hepatocarcinogenesis and liver regeneration was investigated.

METHODS

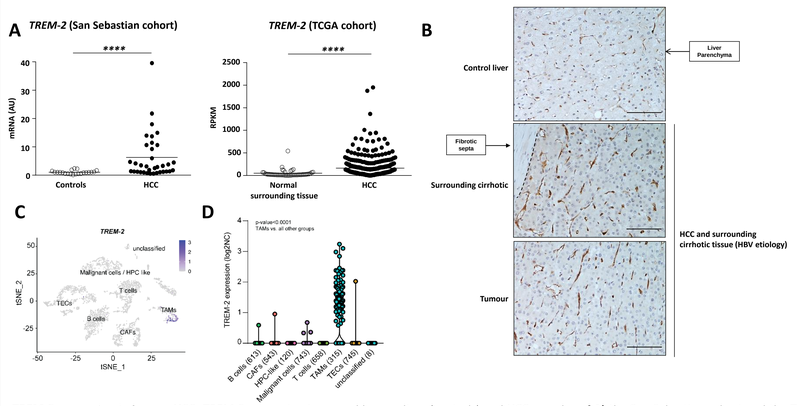
TREM-2 expression was analysed at mRNA and protein level in liver tissue samples of patients with HCC compared to control individuals. To study the role of TREM-2 in HCC, Wild type (WT) and *Trem-2*$^{-/-}$ mice were subjected to experimental models of HCC and liver regeneration. In brief chronic diethylNitrosamine and acute (DEN) models were performed, liver were analyzed at 30 and 40 weeks and 6,24 and 72 h after DEN exposure, respectively. Additionally, the fibrosis associated HCC models DEN coupled to CCl4 and TAA were performed, livers were analyzed at 30 and 40 weeks, respectively. In order to evaluate the role of TREM2 in liver regeneration a ~70% partial hepatectomy (PHx) model was performed and animals were sacrificed at 1, 6, 36, 72 hours and 5 days post-surgery. HCC spheroids were cultured in hanging droplets for 7 days, after which spheroids were transferred to LX-2 conditioned supernatant and spheroid growth was measured.

CONCLUSIONS

- ✓ *TREM-2* expression is upregulated in human HCC and correlates with markers of inflammation
- ✓ The absence of *Trem-2* promotes the development and progression of HCC in mice, regardless of its etiology. In addition, TREM-2 halts liver damage after acute DEN challenge inhibiting inflammation and oxidative stress.
- ✓ TREM-2 halts the initiation of liver regeneration and hepatocyte proliferation following PHx through the inhibition of pro-inflammatory gene expression.
- ✓ The supernatant of human hepatic stellate cells (HSCs) overexpressing TREM-2 inhibits HCC spheroid growth *in vitro*.
- ✓ In view of these data, TREM-2 arises as a potential therapeutic target for HCC.

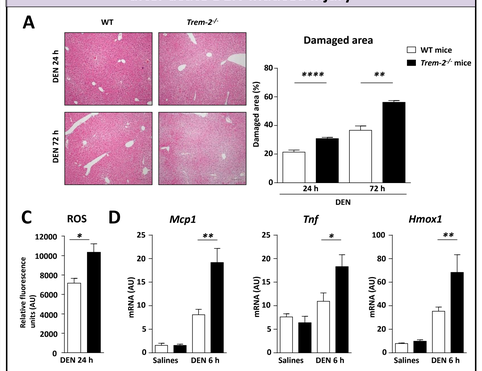
RESULTS

Figure 1
TREM2 is upregulated in human HCC and its expression is mainly restricted to macrophages



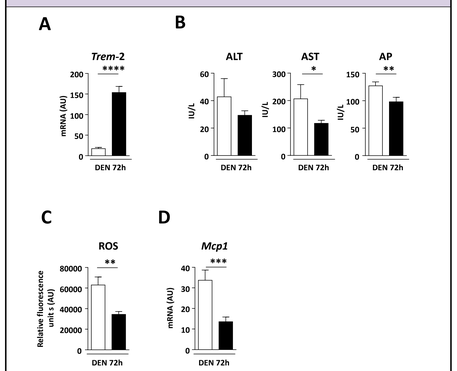
***TREM-2* expression in human HCC.** *TREM-2* expression in normal human liver (controls) and HCC samples of **A)** the San Sebastian cohort and the TCGA cohort of patients. **B)** Representative images of TREM-2 expression by immunohistochemistry in human healthy liver and diseased liver from a patient with an HCC tumor of HBV etiology. **C)** t-SNE plot depicting *TREM-2* expression in TAMs. **D)** and corresponding Log_2 normalized counts (Log_2NC) *TREM-2* expression values for all tumor infiltrating cells types Scale bars represent 100µm.

Figure 2
TREM-2 protects the liver from hepatocyte damage, inflammation and oxidative stress after acute DEN-induced injury



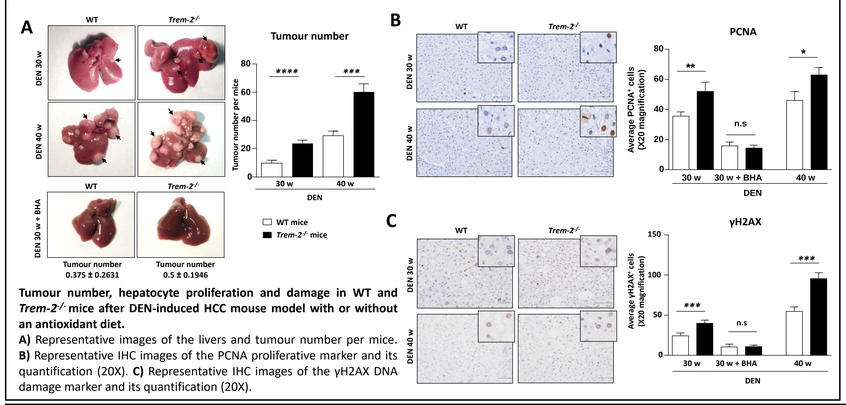
Role of TREM-2 receptor after acute diethylnitrosamine (DEN)-induced liver injury. **A)** Representative H&E and quantification of the damaged area (10X). **C)** ROS levels in the livers of WT and *Trem-2*$^{-/-}$ mice. **D)** Expression levels of the chemokine *Mcp1*, the cytokine *Tnf*, and the oxidative stress enzyme *Hmox1*, 6 hours following DEN.

Figure 3
TREM-2 overexpression lowers liver injury, inflammation and oxidative stress after acute DEN



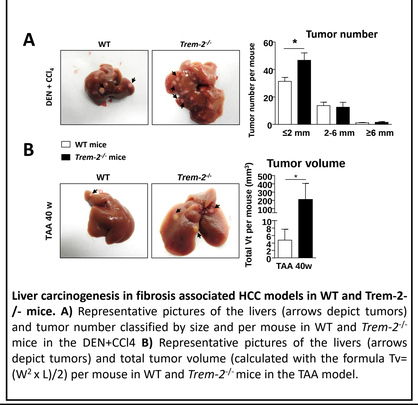
Effects of TREM-2 overexpression after acute DEN-induced liver injury. **A)** Liver TREM-2 mRNA expression at 72h post-DEN. **B)** ALT, AST and AP levels were determined in the serum post-DEN. **C)** ROS was determined in liver tissue of C57BL/6 mice 72 h post-DEN administration with the H2DCFDA dye. **D)** mRNA levels of the chemokine *Mcp1* were determined.

Figure 4
TREM-2 is protective in HCC



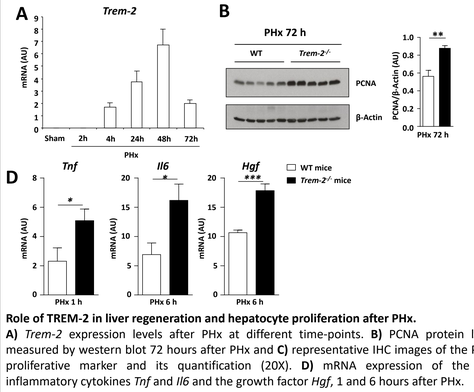
Tumour number, hepatocyte proliferation and damage in WT and *Trem-2*$^{-/-}$ mice after DEN-induced HCC mouse model with or without an antioxidant diet.
A) Representative images of the livers and tumour number per mice. **B)** Representative IHC images of the PCNA proliferative marker and its quantification (20X). **C)** Representative IHC images of the γH2AX DNA damage marker and its quantification (20X).

Figure 6.
***Trem-2* null mice exhibit elevated carcinogenesis in fibrosis-associated HCC**



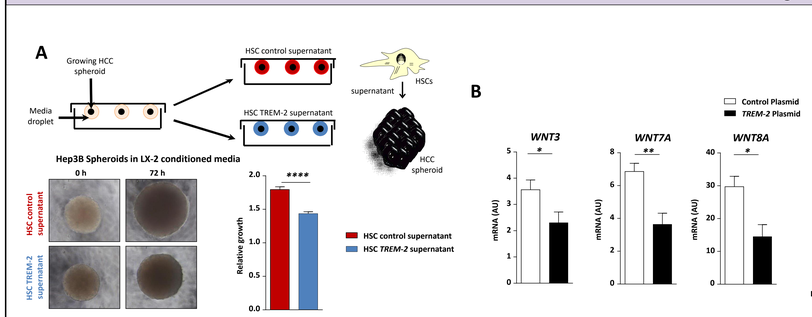
Liver carcinogenesis in fibrosis associated HCC models in WT and Trem-2-/- mice. **A)** Representative pictures of the livers (arrows depict tumors) and tumor number classified by size and per mouse in WT and *Trem-2*$^{-/-}$ mice in the DEN+CCl4 **B)** Representative pictures of the livers (arrows depict tumors) and total tumor volume (calculated with the formula Tv= (W^2 x L)/2) per mouse in WT and *Trem-2*$^{-/-}$ mice in the TAA model.

Figure 7
TREM-2 halts the initiation of liver regeneration and hepatocyte proliferation following PHx



Role of TREM-2 in liver regeneration and hepatocyte proliferation after PHx.
A) *Trem-2* expression levels after PHx at different time-points. **B)** PCNA protein levels measured by western blot 72 hours after PHx and **C)** representative IHC images of the PCNA proliferative marker and its quantification (20X). **D)** mRNA expression of the pro-inflammatory cytokines *Tnf* and *Il6* and the growth factor *Hgf*, 1 and 6 hours after PHx.

Figure 8
TREM-2 reduces HCC tumourigenicity in a paracrine Wnt-dependent manner within HSCs



HCC spheroid culture in LX-2 supernatant. **A)** Hep3B or PLC/PRF5 HCC cells were seeded in hanging droplets for 7 days and formed spheroids were transferred to LX-2 supernatant (LX-2 overexpressing a control plasmid or TREM-2 supernatant) and spheroid size was recorded (20X). **B)** mRNA levels of the canonical WNT ligands *WNT3*, *WNT7A* and *WNT8A* in LX-2 cells overexpressing a control plasmid or TREM-2. **C)** HCC spheroids treated with the Wnt/β-catenin inhibitor IWR-1 and **D)** LX-2 cells or HCC spheroids treated with the WNT ligand secretion inhibitor IWP-2, spheroids were incubated in LX-2 conditioned supernatant and spheroid growth was recorded.

Molecular Pathogenesis, Cell Biology and Translational Research
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P-084
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