Defining the temporal evolution of gut dysbiosis and inflammatory responses leading to hepatocellular carcinoma in Mdr2 -/- mouse model

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BACKGROUND & AIMS

- Emerging evidence implicate the gut microbiome in liver inflammation and hepatocellular carcinoma (HCC) development.
- The Mdr2-/- mouse model captures the stages of inflammation associated HCC seen in humans.
- Using this model, we aimed to characterize the temporal evolution of gut dysbiosis, in relation to the phenotype of systemic and hepatic inflammatory responses leading on to HCC development.

METHODS

- Mdr2-/- mice were used as a model of inflammation-based HCC.
- Time-points were chosen to reflect progression of liver injury leading to HCC development.
- Liver histology was performed to confirm pathological changes across the spectrum of liver injury and to confirm HCC development.
- Gut microbiota composition was analyzed with 16S rRNA sequencing.
- Serum lipopolysaccharide (LPS) and serum cytokines/chemokines were measured with ELISA based immunoassays.
- Intrahepatic genes related to the innate and adaptive immune response were measured with quantitative real time (RT) PCR.

RESULTS

- We confirmed that progression of liver injury and HCC formation to occur at chosen time-points.
- Early stages of liver injury, inflammation and cirrhosis, were characterized by dysbiosis (Fig 1A)
- Microbiota functional pathways pertaining to gut barrier invasion were enriched during the initial phase of liver inflammation, whilst those supporting lipopolysaccharide (LPS) biosynthesis increased as cirrhosis and HCC evolved (Fig 1B)
- In parallel, serum LPS progressively increased during the course of liver injury, corresponding to a shift towards a systemic Th1/Th17 proinflammatory phenotype (Fig 1C)
- With this, intrahepatic inflammatory gene profile transitioned from a proinflammatory phenotype in the initial phases of liver injury to an immunosuppressed one in HCC (Fig 1D)
- In established HCC, a switch in microbiota function from carbohydrate to amino acid metabolism occurred (Fig 1B)

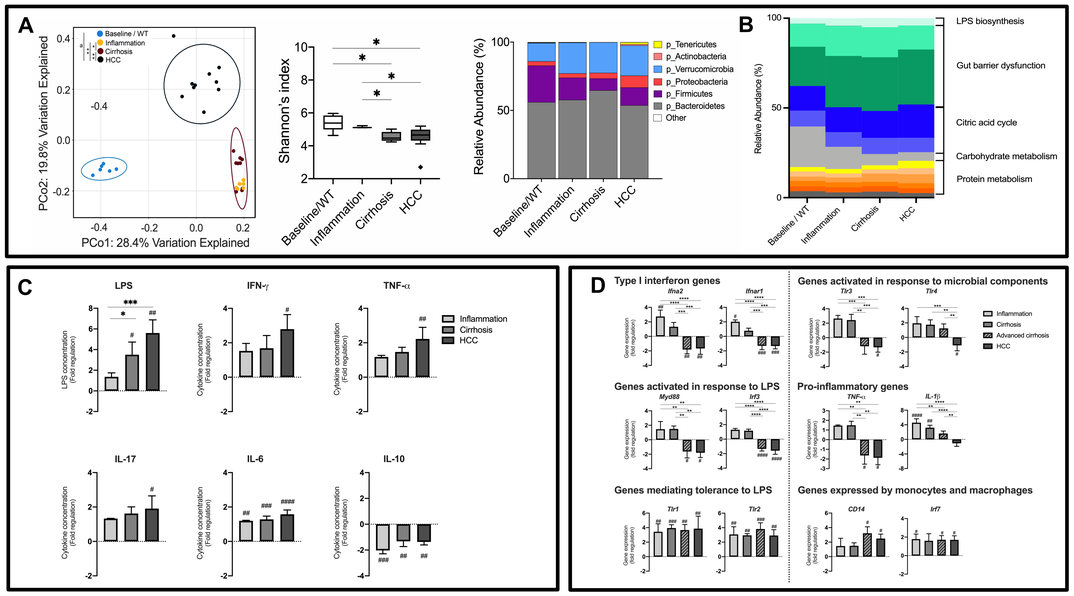

Fig 1. A. Microbiome diversity and taxonomy at each stage of liver disease, showing differences in β-diversity by Bray–Curtis dissimilarity as displayed in the Principal Coordinates Analysis (PCoA), α-diversity by Shannon index and relative taxonomic abundance at the phylum level. **B.** Predicted microbial function assessed by KEGG annotation demonstrating a shift in microbial functions with progression of liver disease. **C.** Serum LPS and cytokine levels demonstrating development of a proinflammatory systemic response with liver disease progression. The baseline/WT time point is represented as the dashed horizontal line, from which fold regulation in LPS or cytokine concentration is calculated **D.** Changes in fold regulation of key genes expressed in liver tissue at various stages of liver injury/disease. Advanced cirrhosis represents the peritumoural tissue at 42 weeks, whilst HCC represents the tumor tissue proper at 42 weeks. The baseline/WT time point is represented as the dashed horizontal line, from which fold regulation in gene expression is calculated. *P*-value calculated by One-way ANOVA with Turkey's multiple comparisons test, with * representing difference between groups and # representing difference compared to baseline/WT time point. $^{**}P < 0.01$; $^{***}P < 0.001$; $^{****}P < 0.0001$ and $^{\#}P < 0.05$; $^{\#\#}P < 0.01$ and $^{\#\#\#}P < 0.001$.

CONCLUSIONS

- In Mdr2-/- mice, dysbiosis preceded HCC development, with temporal evolution of microbiota composition and function in a manner supporting gut invasion, LPS biosynthesis, and redirection of energy source utilization (Fig 2)
- Corresponding to these microbial events, a transitional shift in systemic and intrahepatic inflammatory responses occur supporting HCC development (Fig 2)
- These findings support the notion that gut based therapeutic interventions could be beneficial early in the course of liver disease to halt HCC development

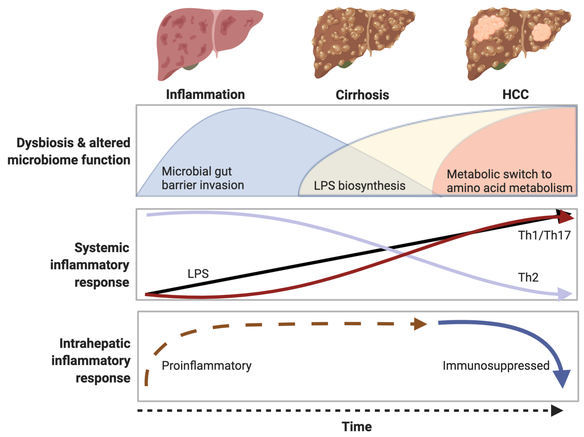

Fig 2. Schema summarizing key findings. The temporal evolution of the gut microbiome, its functionality and inflammatory responses in Mdr2 -/- model of HCC

CONTACT INFORMATION AND ACKNOWLEDGEMENTS

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