# Altered transcriptional regulation underlying alcohol induced liver cirrhosis in a porcine hepatocellular carcinoma model

**Alvi Yasmin[1], Daniel P. Regan[2], Lawrence B. Schook[1,3], Ron C. Gaba[1], Kyle M. Schachtschneider[1,4,5]**

[1]Department of Radiology, University of Illinois at Chicago, Chicago, IL
[2]Flint Animal Cancer Center, Colorado State University
[3]Department of Animal Sciences, University of Illinois at Urbana-Champaign
[4]Department of Biochemistry and Molecular Genetics, University of Illinois at Chicago
[5]National Center for Supercomputing Applications, University of Illinois at Urbana-Champaign

University of Illinois Hospital & Health Sciences System — Radiology

## Introduction

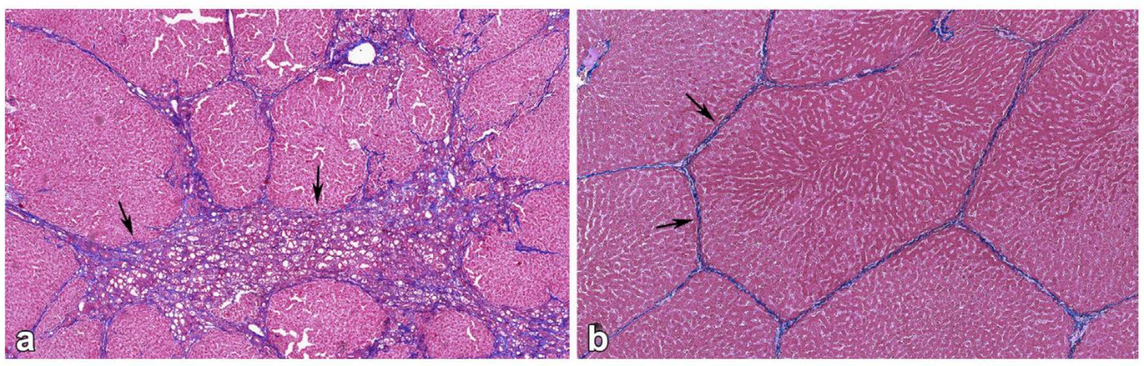
- Hepatocellular carcinoma (HCC) is the 2nd deadliest cancer that accounts for more than 9% cancer death annually.
- 25% of HCC cases are associated with alcohol induced liver cirrhosis, which prompts hepatic inflammation, cell necrosis, and fibrosis deposition.
- Development of a large animal model capable of exhibiting both HCC and alcohol induced liver cirrhosis is essential for understanding the effects of the cirrhotic liver microenvironment on HCC tumor biology and therapeutic response.
- This study utilized the Oncopig Cancer Model (OCM)—a transgenic pig model that recapitulates human HCC through induced expression of *KRAS*$^{G12D}$ and *TP53*$^{R167H}$ transgenes—to investigate the molecular mechanisms underlying alcohol induced liver cirrhosis induction and reversal.

## Materials and Methods

- Oncopigs (n=5) underwent cirrhosis induction via infusion of ethanol and ethiodized oil (1:3 v/v dosed at 0.75 mL/kg) into the hepatic arterial circulation, which results in transient fibrosis that peaks in severity at 8 weeks post induction and reverts to normal by 20 weeks post induction[1].
- OCM cirrhotic and healthy age matched control (n=5) liver tissue was harvested 8 week post induction and gene expression profiled via RNA-seq.
- Differentially expressed genes (DEGs) were identified using DESeq2.
- GO term enrichment analysis was performed using the Gene Ontology Consortium.

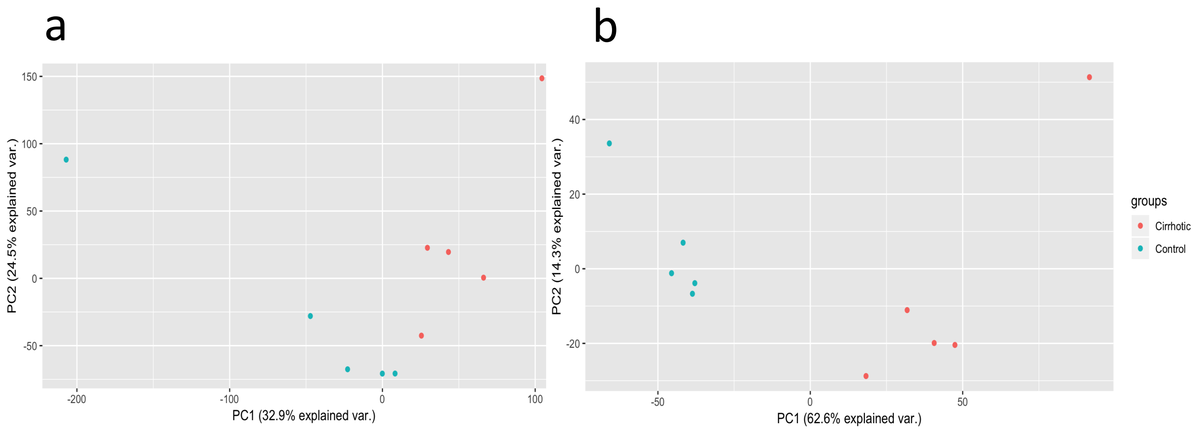
## Results

### Figure 1. Alcohol exposure results in fibrosis induction in OCM livers




Representative images of Masson's trichrome-stained OCM liver sections histologically graded for fibrosis using a porcine adapted METAVIR scheme. (a) F3 fibrosis with numerous dissecting septa 8 weeks post-induction. (b) Control, histologically normal OCM liver with normal pre-existing fibrous septa. Histopathological analysis of liver samples demonstrated the METAVIR fibrosis score for cirrhotic liver (median F3, range F2-F4) was significantly higher than control liver samples (median F0, range F0-F1) at 8 weeks ($P$ = 0.010).

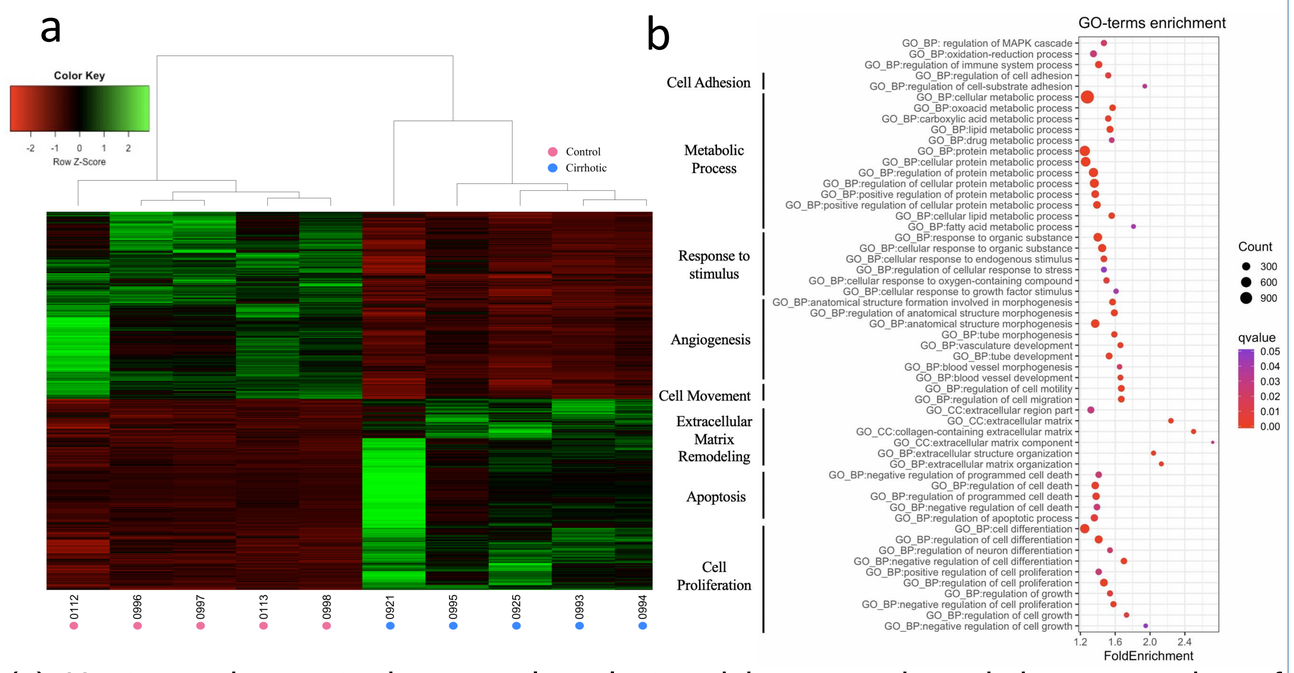
### Figure 2. Alcohol exposure results in reproducible alteration of genome-wide and differential hepatic gene expression profiles




PCA based on the relative expression of (a) 21,748 known genes for which expression information was available for each sample and (b) 4,387 DEGs resulted in cirrhotic and control samples clustering by group. PCA plots indicate that significant and consistent changes in hepatic gene expression were induced by alcohol exposure in the cirrhotic liver (ANOSIM R = 0.248, P = 0.006; ANOSIM R = 0.788, P = 0.011; respectively).

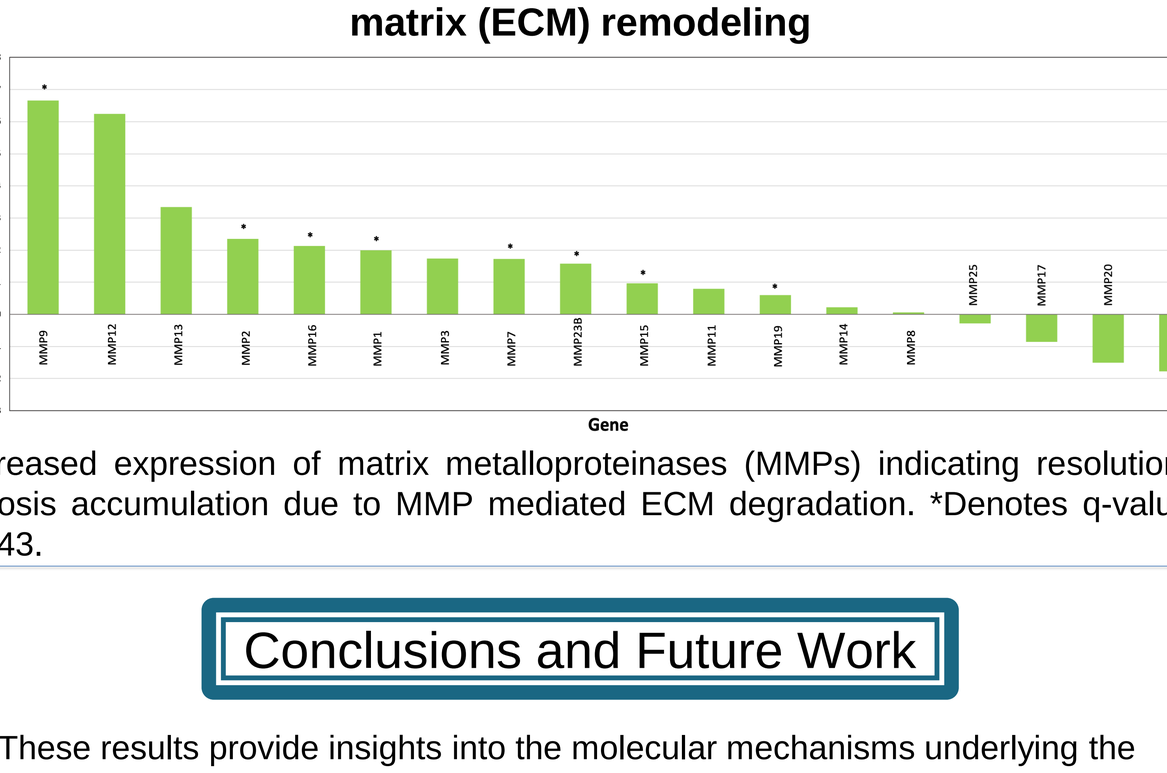
## Results (cont.)

### Figure 3. GO term enrichment analysis identifies biological processes associated with alcoholic liver disease in humans



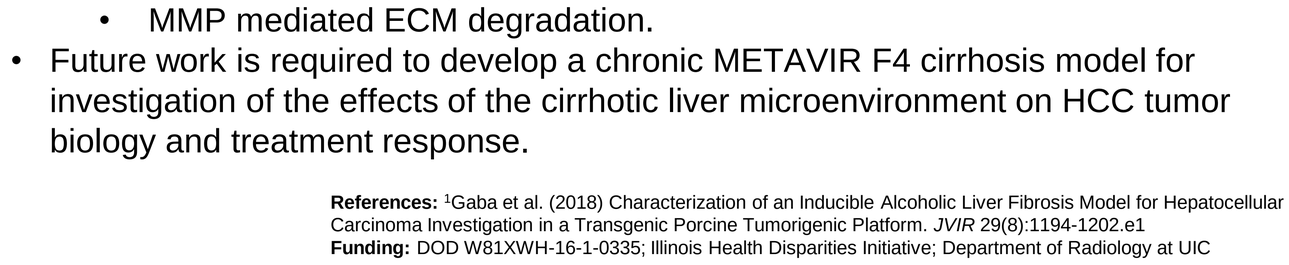

(a) Heatmap demonstrating samples clustered by group based the expression of 4,387 identified DEGs. (b) GO terms significantly enriched for DEGs associated with liver cirrhosis progression in humans.

### Figure 4. Downregulation of *MYC* associated with inactivation of hepatic stellate cells (HSCs)




(a) Reduced expression of *MYC* was observed in the OCM cirrhotic liver samples (log2 fold change = -1.68, q-value = 9.5x10$^{-4}$). (b) As upregulation of *MYC* results in HSC activation, hepatic apoptosis, and increased inflammation, downregulation of *MYC* suggests fibrosis deposition is no longer occurring 8 weeks post induction. *Denotes q-value = 9.5x10$^{-4}$

### Figure 5. Increased expression of major regulators of extracellular matrix (ECM) remodeling




Increased expression of matrix metalloproteinases (MMPs) indicating resolution of fibrosis accumulation due to MMP mediated ECM degradation. *Denotes q-value < 0.043.

## Conclusions and Future Work

- These results provide insights into the molecular mechanisms underlying the transient nature of the OCM cirrhosis model:
  - Reduced fibrosis deposition due to inactivation of HSCs.
  - MMP mediated ECM degradation.
- Future work is required to develop a chronic METAVIR F4 cirrhosis model for investigation of the effects of the cirrhotic liver microenvironment on HCC tumor biology and treatment response.

**References:** [1]Gaba et al. (2018) Characterization of an Inducible Alcoholic Liver Fibrosis Model for Hepatocellular Carcinoma Investigation in a Transgenic Porcine Tumorigenic Platform. *JVIR* 29(8):1194-1202.e1
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