Characterization of an inducible alcoholic liver fibrosis model for hepatocellular carcinoma investigation in a transgenic porcine platform

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Introduction

- Hepatocellular Carcinoma (HCC) is a deadly tumor that spans more than 780,000 new diagnoses and causes 750,000 annual deaths worldwide.
- Alcoholic liver disease represents a common progressive chronic liver ailment that incites liver cirrhosis—a precancerous state of liver scarring—that increases the risk for HCC development
- The health status of the liver affects HCC tumor biology and treatment responses; therefore, a large animal model capable of exhibiting both HCC and alcohol induced liver cirrhosis would be a valuable resource for preclinical research investigating HCC in its native comorbid cirrhotic microenvironment.
- Pigs represent ideal human disease models due to their similar size, anatomy, metabolism, genetics, and epigenetics compared to humans.
- This study utilized the innovative Oncopig Cancer Model (OCM)-–a transgenic swine model that recapitulates human cancer through development of site and cell specific tumors via induced expression of heterozygous *KRAS*^{G12D} and *TP53*^{R167H} transgenes—to develop alcohol induced fibrosis in a porcine model capable of developing HCC tumors.

Materials and Methods

- Liver injury was induced in Oncopigs (Cohort 1 n=5; Cohort 2 n=5) via transcatheter infusion of absolute ethanol and ethiodized oil (1:3 v/v dosed at 0.75 mL/kg) into the hepatic arterial circulation.

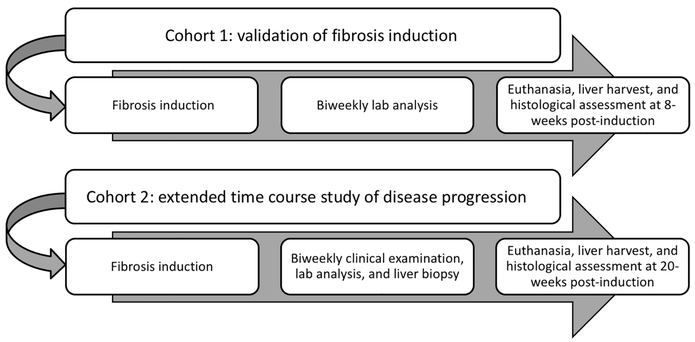
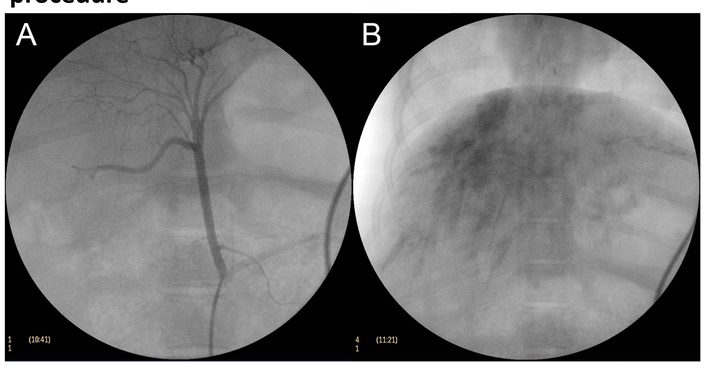


Table 1. Porcine adapted modified METAVIR fibrosis grading scheme

Grade	Description
F0	Normal porcine liver; no increase in fibrosis
F1	Mild fibrous expansion of portal areas and/or mild thickening/expansion of few random segments of normal pre-existing fibrous septa
F2	Mild to moderate fibrous expansion of portal tracts and multiple, random, non-contiguous segments of normal fibrous septa surrounding multiple hepatic lobules ± presence of thin bands of fibrosis extending from septa or portal tracts into adjacent lobular parenchyma
F3	Moderate to marked fibrous expansion of contiguous segments of fibrous septa surrounding multiple hepatic lobules; fibrous expansion can involve contiguous segments of septa, and partially encircle hepatic lobules, but typically does not completely circumscribe lobules. Presence of fibrous connective tissue which dissects into lobular parenchyma, surrounding and separating cords of hepatocytes
F4	Cirrhosis; normal fibrous septa surrounding hepatic lobules are expanded by moderate to marked amounts of fibrous connective tissue, with some portal bridging, and frequent dissection into adjacent lobular parenchyma, and separation of hepatic cords. Fibrous connective tissue often completely circumscribes multiple hepatic lobules, which appear irregular/shrunken.

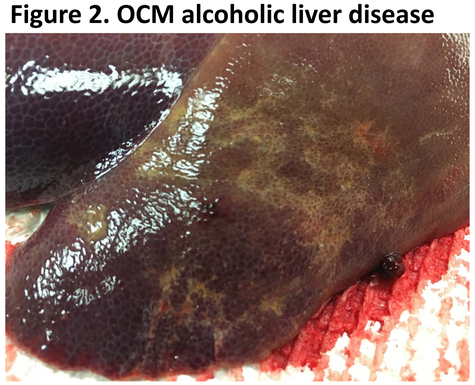
Results

Figure 1. Representative images from fibrosis induction procedure



A. Celiac arteriogram demonstrates conventional porcine hepatic arteries. B. Fluoroscopic image obtained after administration of ethanol and ethiodized oil demonstrates deposition of radiopaque emulsion throughout liver.

Figure 2. OCM alcoholic liver disease



Gross pathology of OCM liver 8-weeks post-induction demonstrates undulation and nodularity of hepatic capsular surface, enhanced reticular pattern, and discoloration of hepatic parenchyma consistent with macronodular fibrosis.

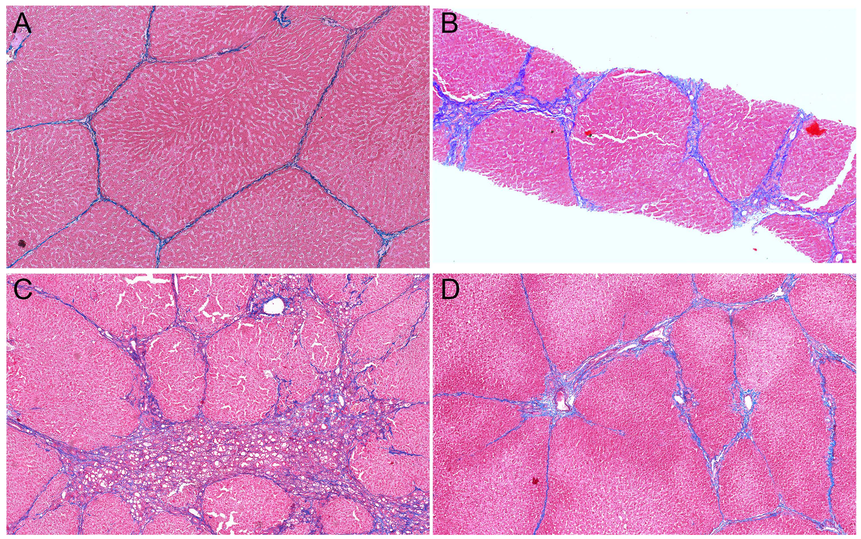
Results (cont.)

Table 2. METAVIR Fibrosis and Inflammation Classifications

	2 Weeks Post Induction	8 Weeks Post Induction	Age Matched Controls	20 Weeks Post Induction
Inflammation	A1 to A2	A2 to A3	A0 to A1	A1
METAVIR	F1 to F3	F2 to F4	F0 to F1	F1 to F2
Fibrosis %	4.1	15.3	8.7	9.7

OCM liver specimens staged for fibrosis and inflammation classification according to a porcine adapted METAVIR system (n=5/time point). Percent fibrosis was quantified using trichrome stained slides.

Figure 3. Representative images of Masson's trichrome stained OCM livers



Representative images of Masson's trichrome stained OCM liver sections histologically graded for fibrosis using a porcine adapted METAVIR scheme (all 5x magnification). A. Control, histologically normal OCM liver with normal pre-existing fibrous septa. B. METAVIR F2 score 2 weeks post induction. C. METAVIR F3 score 8 weeks post induction. D. METAVIR F2 score 20 weeks post induction.

Conclusions and Future Work

- This study successfully validated a protocol to develop a pre-cirrhotic METAVIR F2-F3 model of alcoholic liver disease within 8 weeks using transarterial ethanol-ethiodized oil infusion.
- Induction of alcoholic liver injury resulted in METAVIR stage F2-F4 fibrosis, stage A2-A3 inflammation, and elevated fibrosis levels compared to age matched controls after 8 weeks.
- METAVIR stage F1-F3 fibrosis was observed as early as 2 weeks post induction.
- As observed clinically for patients presenting with pre-cirrhotic alcohol induced liver damage, a lack of persistent alcohol exposure resulted in liver recovery based on temporal profiling up to 20 weeks post induction.
- No behavioral or biochemical abnormalities were observed during the monitoring period to suggest liver decompensation.
- These results support the OCM's capability to serve as a model for HCC in a cirrhotic liver background.
- In an effort to develop an irreversible METAVIR stage F4 porcine model of cirrhosis, further studies will closely mimic human alcoholic liver disease in two important ways: (1) prolonged and repetitive exposure of the liver parenchyma to alcohol, and (2) simultaneous administration of an immune antigen to catalyze immune-mediated biochemical processes involved in cirrhosis.

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