LABORATORY INVESTIGATION

Characterization of an Inducible Alcoholic Liver Fibrosis Model for Hepatocellular Carcinoma Investigation in a Transgenic Porcine Tumorigenic Platform

Ron C. Gaba, MD, Nasya Mendoza-Elias, BS, Daniel P. Regan, DVM, PhD, Kelly D. Garcia, DVM, PhD, R. Peter Lokken, MD, MPH, Regina M. Schwind, BS, Michael Eichner, DVM, Faith M. Thomas, BS, Lauretta A. Rund, PhD, Lawrence B. Schook, PhD, and Kyle M. Schachtschneider, PhD

ABSTRACT

Purpose: This study used the Oncopig Cancer Model (OCM) to develop alcohol-induced fibrosis in a porcine model capable of developing hepatocellular carcinoma.

Materials and Methods: Liver injury was induced in 8-week-old Oncopigs (n = 10) via hepatic transarterial infusion of 0.75 mL/kg ethanol-ethiodized oil (1:3 v/v). Feasibility was assessed in an initial Oncopig cohort (n = 5) by histologic analysis at 8 weeks after induction, and METAVIR results were compared to age- and sex-matched healthy controls (n = 5). Liver injury was then induced in a second OCM cohort (n = 5) for a time-course study, with post-induction disease surveillance via biweekly physical exam, lab analysis, and liver biopsies until 20 weeks after induction.

Results: In Cohort 1, 8-week post-induction liver histologic analysis revealed median METAVIR F3 (range, F3–F4) fibrosis, A2 (range, A2–A3) inflammation, and 15.3% (range, 5.0%–22.9%) fibrosis. METAVIR and inflammation scores were generally elevated compared to healthy controls (F0–F1, $P = 0.0013$; A0–A1, $P = .0013$; median percent fibrosis 8.7%, range, 5.8%–12.1%, $P = .064$). In Cohort 2, histologic analysis revealed peak fibrosis severity of median METAVIR F3 (range, F2–F3). However, lack of persistent alcohol exposure resulted in liver recovery, with median METAVIR F2 (range, F1–F2) fibrosis at 20 weeks after induction. No behavioral or biochemical abnormalities were observed to indicate liver decompensation.

Conclusions: This study successfully validated a protocol to develop METAVIR F3–F4 fibrosis within 8 weeks in the OCM, supporting its potential to serve as a model for hepatocellular carcinoma in a fibrotic liver background. Further investigation is required to determine if repeated alcohol liver injury is required to develop an irreversible METAVIR grade F4 porcine cirrhosis model.

ABBREVIATIONS

AST = aspartate aminotransferase, HCC = hepatocellular carcinoma, LRT = locoregional therapy, MRE = magnetic resonance elastography, OCM = Oncopig Cancer Model

Hepatocellular carcinoma (HCC) is a deadly tumor that accounts for more than 9% of annual cancer mortality (1,2). While global cancer incidence is generally decreasing, HCC incidence is projected to continually increase for the foreseeable future, given the growing prevalence of chronic liver diseases that cause liver cirrhosis (3). Alcoholic liver disease is a major cause of liver cirrhosis and increases the risk for hepatocarcinogenesis (4). Since the health status of

From the Department of Radiology (R.C.G., R.P.L., R.M.S., L.B.S., K.M.S.), University of Illinois Health, 1740 West Taylor Street, MC 931, Chicago, Illinois, 60612; College of Medicine (N.M-E.) and Biological Resources Laboratory (K.D.G., M.E.), University of Illinois at Chicago, Chicago, Illinois; Flint Animal Cancer Center (D.P.R.), Colorado State University, Fort Collins, Colorado; and Department of Animal Sciences (F.M.T., L.A.R., L.B.S), University of Illinois at Urbana-Champaign, Urbana and Champaign, Illinois. Received January 16, 2018; final revision received March 3, 2018; accepted March 7, 2018. **Address correspondence to** K.M.S.; E-mail: kschach2@uic.edu

None of the authors have identified a conflict of interest.

Appendix A and Table E1 are available online at *www.jvir.org*.

https://doi.org/10.1016/j.jvir.2018.03.007

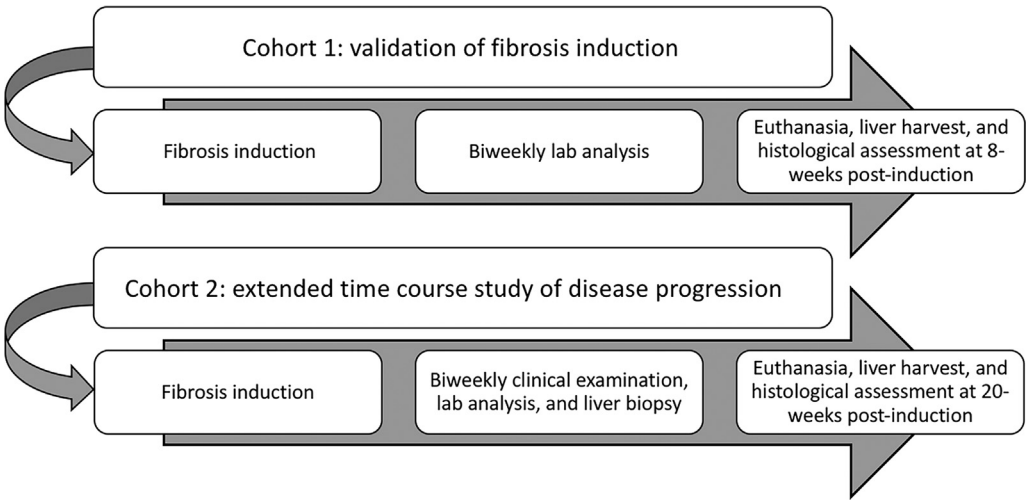

Figure 1. Flow chart illustrating study design schema. Pathologic outcomes in Cohort 1 were also compared to age- and sex-matched healthy controls.

the liver can also have profound effects on HCC tumor biology, treatment allocation, and response to therapy (5), a large animal model capable of exhibiting both HCC and liver cirrhosis concurrently would be a valuable resource for advancing preclinical investigation of HCC detection, development, natural history, and response to treatment in its native comorbid cirrhotic background. Although previous studies have investigated cirrhosis induction in domestic pigs (6), the applicability of induction protocols across genotypically distinct pig breeds, including those amenable to tumor development, is unknown. This study used the innovative Oncopig Cancer Model (OCM)—a transgenic porcine model that recapitulates human cancer through development of site- and cell-specific tumors via induced expression of heterozygous $KRAS^{G12D}$ and $TP53^{R167H}$ transgenes (7)—to develop alcohol-induced fibrosis in a porcine model capable of developing HCC tumors (8).

MATERIALS AND METHODS

Animal Subjects and Study Design

Work was completed at the University of Illinois at Chicago and the University of Illinois at Urbana-Champaign. Institutional Animal Care and Use Committee approval was obtained. Details of animal care are in **Appendix A** (available online at *www.jvir.org*). The study design consisted of 2 chronologically staged experiments spanning 2 successive cohorts of non-tumor-laden Oncopigs (**Fig 1**). Sample sizes reflected those typically used in exploratory, pilot studies (9). The first experiment aimed to validate the ability of a previously published protocol (6) to produce liver fibrosis in the OCM. In the first OCM cohort (n = 5), liver injury was induced and followed by post-induction disease surveillance via biweekly lab analysis, with subsequent euthanasia, liver harvest, and liver histologic assessment at 8 weeks after induction. Laboratory and histologic results were also compared to age- and sex-matched healthy control Oncopigs (n = 5). Liver was harvested from control animals for pathologic comparison at age-matched time points to subjects in Cohort 1. After validation of fibrosis induction in Oncopigs, the procedure was repeated for an extended time-course experiment in a second OCM cohort (n = 5) to investigate disease progression and the ability to provoke sustained liver disease. In this second OCM cohort, physical examination, lab analysis, and serial liver biopsies were obtained at 2-week intervals for 20 weeks, followed by euthanasia, liver harvest, and histologic assessment.

Study Cohort

The study cohort included 10 female Oncopigs of a median age of 57 days (range, 51–62 days) and a median weight of 14.0 kg (range, 11.7–18.2 kg) at the time of fibrosis induction. Oncopigs in Cohort 1 were significantly older (median age, 62 days; range, 60–62 days versus 54 days; range, 51–54 days; $P = .008$) and heavier (median weight, 16.2 kg; range, 15.9–18.2 kg versus 12.4 kg, range, 11.7–14.2 kg; $P = .036$) than those in Cohort 2 at the time of fibrosis induction. No significant differences were observed in biometric data between the Oncopigs in Cohort 1 and the healthy age- and sex-matched control subjects (median weight, 36.9 kg; range, 26.5–42.7 kg versus 39.0 kg; range, 35.8–40.4 kg; $P = .841$) at 16 weeks of age (corresponding to 8 weeks after induction).

Procedures

Fibrosis Induction.—All fibrosis induction procedures were performed by 1 of 2 board-certified interventional radiology (IR) physicians with 9 years of experience (R.C.G.) and 4 years of experience (R.P.L.), according to a modification of the methodology described by Avritscher et al (6). At 8 weeks of age, Oncopigs underwent anesthetic induction, followed by intubation and maintenance with 1%–3% isoflurane. Angiography was performed using a C-arm (OEC Medical Systems series 9600; GE Healthcare, United Kingdom). With the animal supine, the groin was sterilely prepared. Ultrasound-guided vascular access was gained via