Development of Porcine Hepatocellular Carcinoma Cell Lines: Comprehensive *in vitro* and *in vivo* Characterization

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Abstract #2776

Introduction

- Hepatocellular carcinoma (HCC) spans more than 780,000 new annual diagnoses & causes 750,000 yearly mortalities
- Preclinical animal models represent pivotal tools for translational investigations to develop & test novel therapeutics for HCC both *in vitro* & *in vivo*
- Development of clinically relevant systems to serve as a bridge between preclinical murine studies & human clinical practice is of vital importance
- The Oncopig Cancer Model (OCM) is a novel transgenic swine platform that recapitulates human cancer through development of site/cell specific tumors after Cre recombinase induced expression of heterozygous *KRAS*^{G12D} & *TP53*^{R167H} transgenes.
- In this study, we tested the hypothesis that isolation & transformation of OCM hepatocytes from multiple individuals results in development of phenotypically consistent porcine HCC (pHCC) cell lines which faithfully recapitulate the *in vitro* & *in vivo* features of human HCC.

Materials & Methods

- Fourteen pHCC lines were established from primary hepatocytes isolated from resected liver specimens (median 10.0, range 4.9-26.0 g) of 4- to 8-week-old OCMs (n = 14), with a median yield of 3.1 x 10^6 (range 7.0 x 10^5-1.3 x 10^7) cells/g & 57% (range 20-97%) viability.
- At 24-hours post-isolation, porcine hepatocytes were transformed into pHCC using Ad-Cre-green fluorescent protein (GFP) (Fig. 1) with median 92% (range 70-99%) efficiency, & were maintained in culture for median 11 (range 7-15) passages.
- Morphological & behavioral phenotyping of pHCC cells performed using qualitative & quantitative assays were compared to the most widely used human HCC cell line for *in vitro* investigations (HepG2)
- pHCC *in vivo* malignant potential was evaluated in SCID mice & OCM donors

Transformation of Porcine Hepatocytes to pHCC

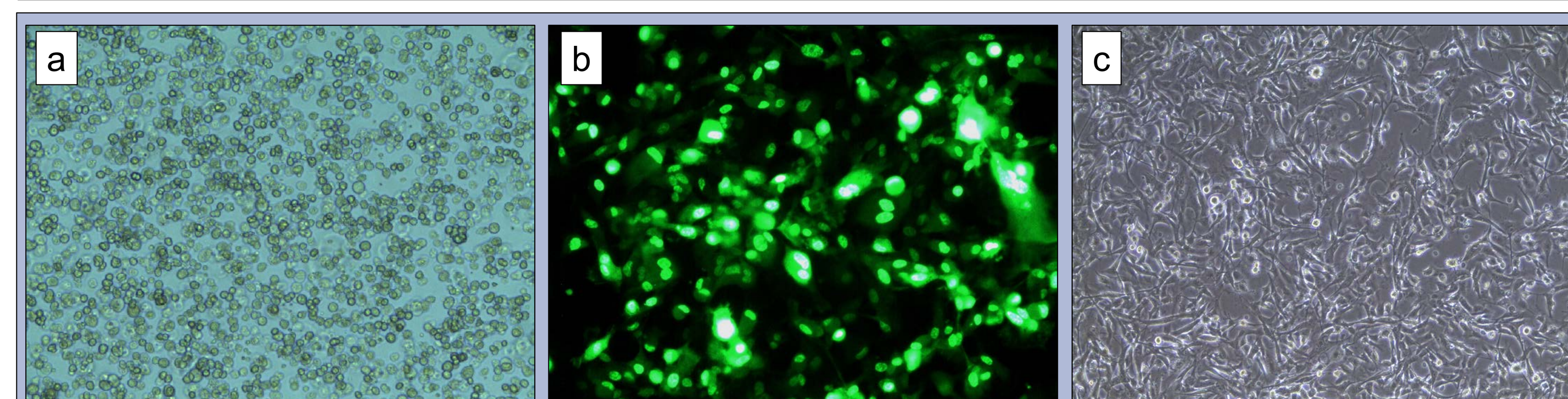


Figure 1. Representative photographs display (a) primary hepatocytes after isolation; (b) transformed hepatocytes displaying GFP; & (c) adherent pHCC cells (magnification 10x)

KRAS^{G12D} & *TP53*^{R167H} Transgene Expression

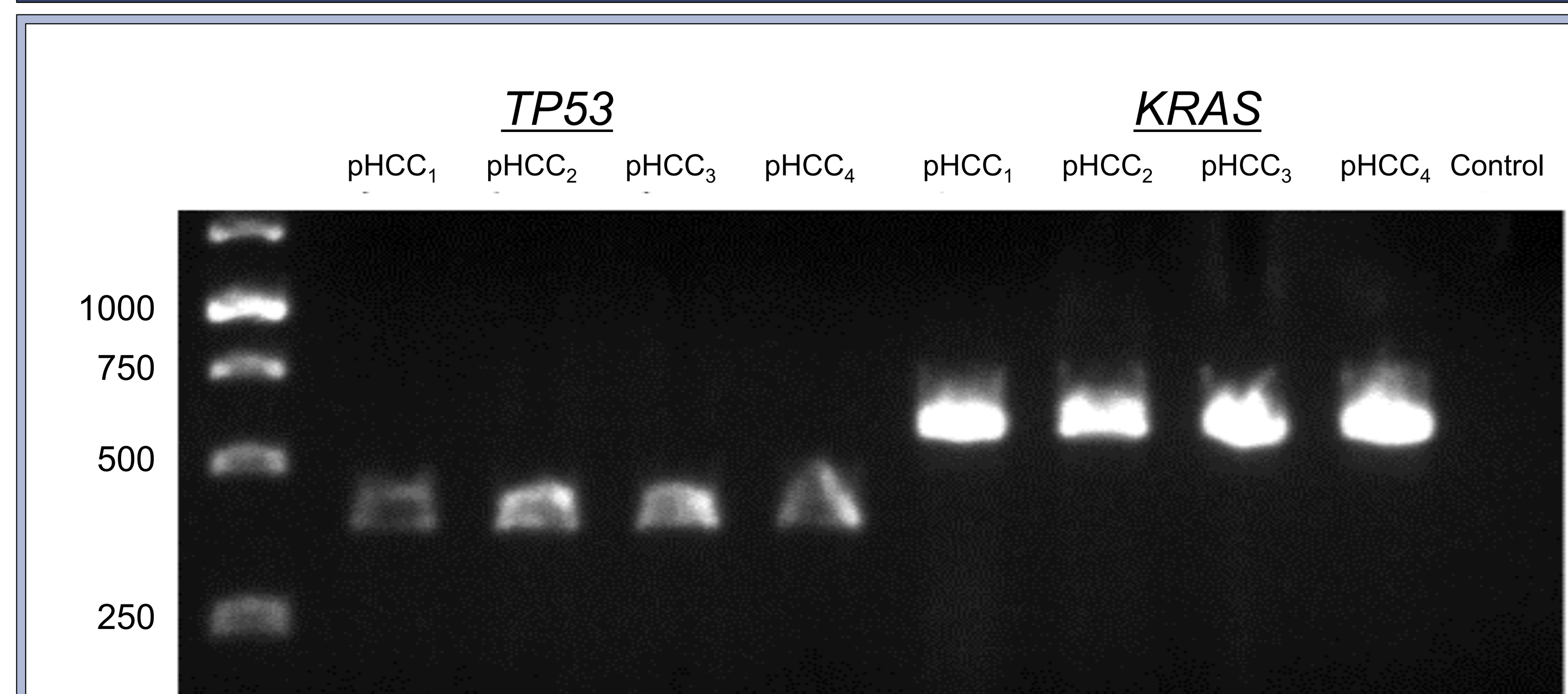


Figure 2. Fourteen of 14 (100%) pHCC cell lines showed RT-PCR proven transgene expression on agarose gel electrophoresis, confirming malignant transformation. Above gel shows four representative cell lines.

Immunohistochemistry (IHC)

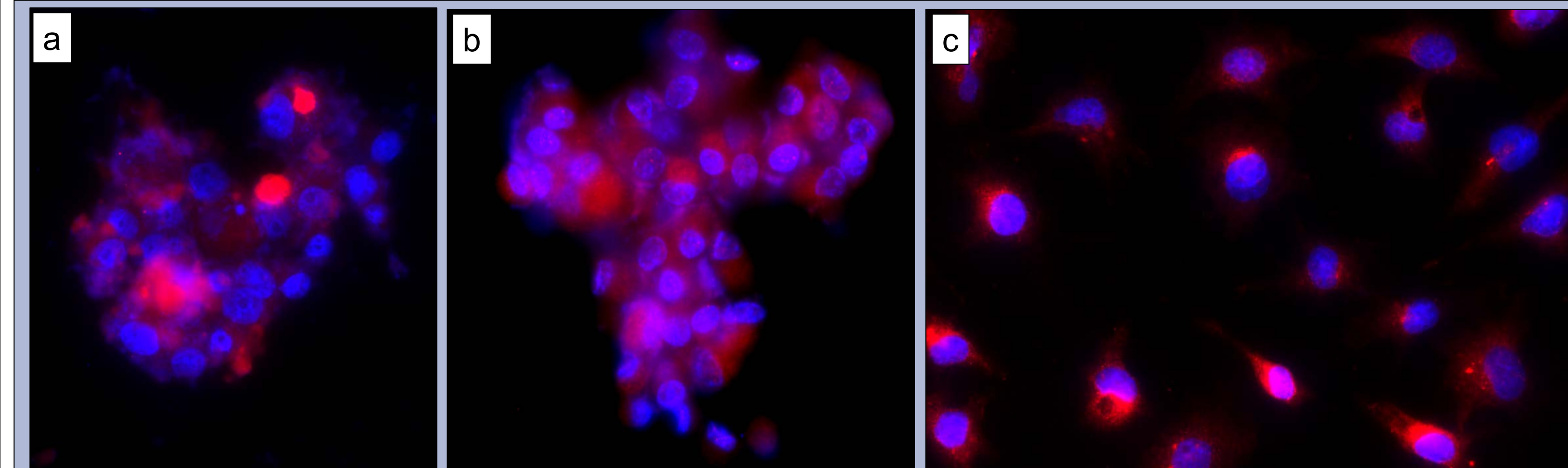


Figure 3. Similar to human HCC, all pHCC cell lines exhibited Arginase-1 IHC positivity, indicating hepatocellular origin. Photographs display (a) & (b) pink cytoplasmic staining of representative pHCC cells with liver specific marker Arg-1; (c) HepG2 cells show similar staining pattern

Cell Cycle Length

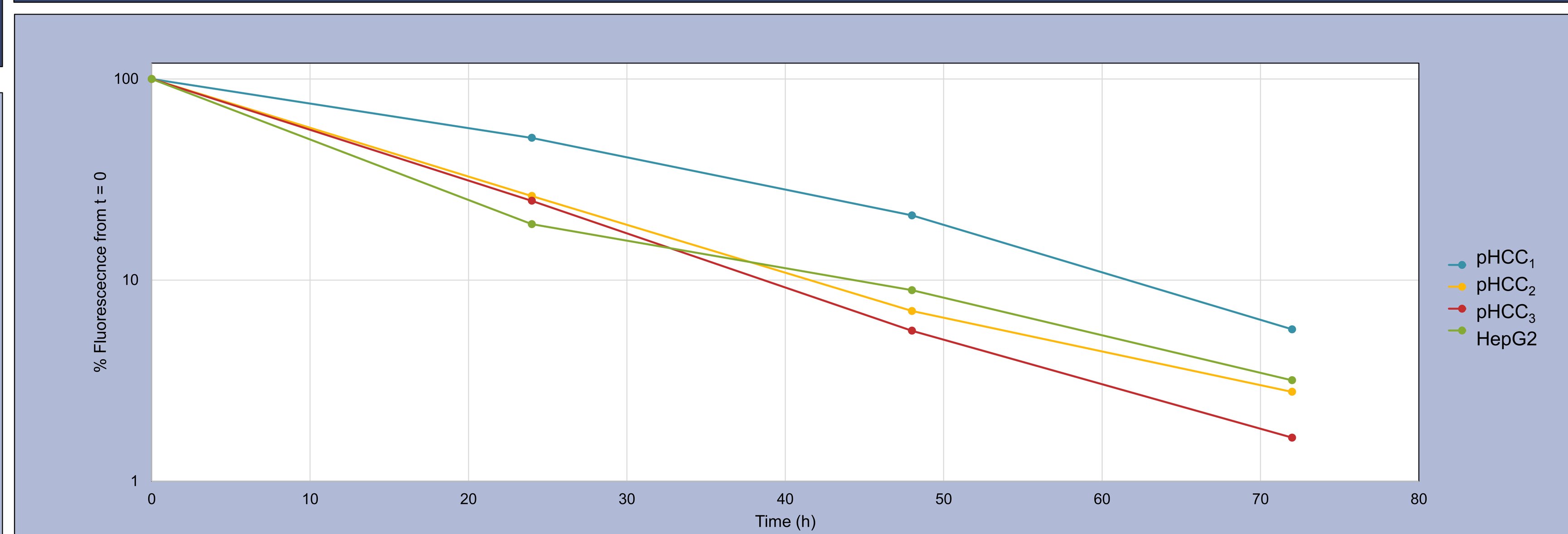


Figure 4. Cells stained with CFSE dye & tested for fluorescence at 0 h, 24 h, 48 h, & 72 h. Plot of median fluorescence intensity for 3 representative pHCC cell lines & HepG2 shows similar cell cycle length. Median pHCC cell cycle length was 13.5 (range 10.0-16.9) hours, similar to human HCC (15.1 hours).

Migration Assay

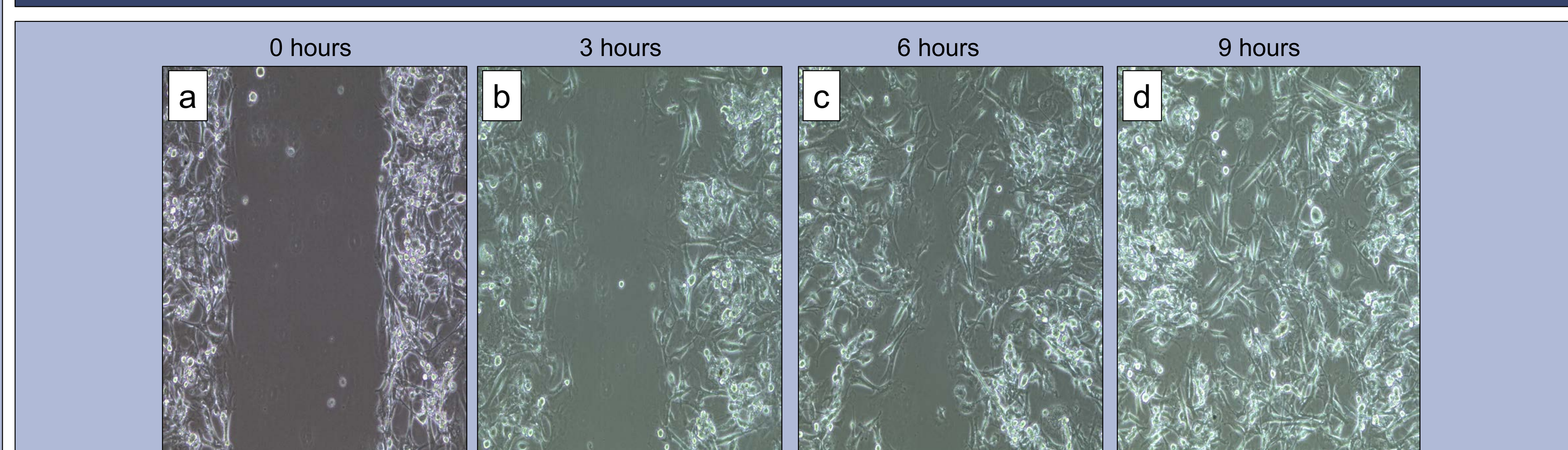
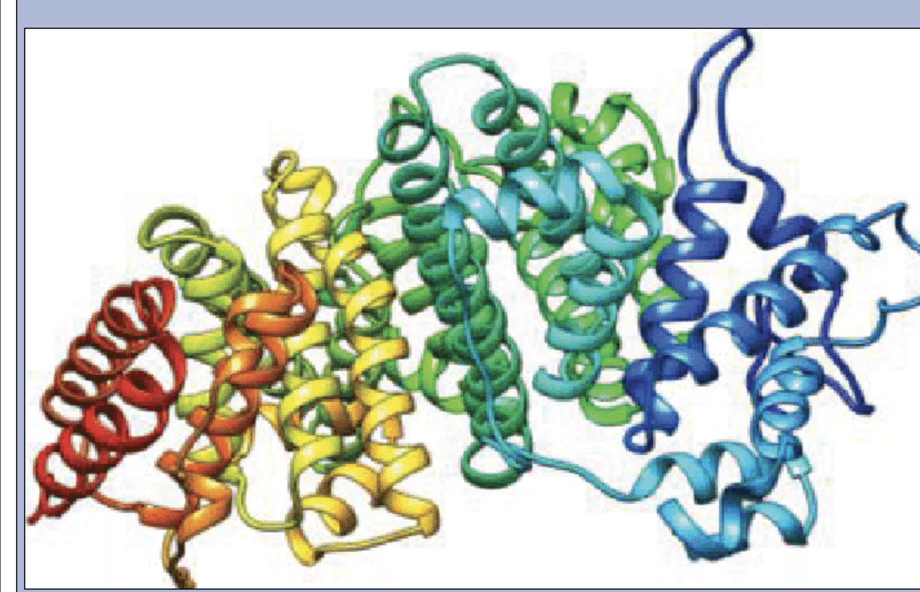


Figure 5. For the migration assay, cells were grown in a culture-insert 2 well plate (Ibidi) for 24 h, & the inter-cellular gap distance was measured within 0 h, 4 h, 8 h, & 24 h. Testing was performed in triplicate. Representative photographs (a-d) from pHCC migration assay demonstrate progressive gap closure. Median time to half gap closure for all pHCC cell lines was 7.5 (range 4.1-20.9) h, comparable to HepG2 (3 hours).

α-fetoprotein (AFP) Production



AFP is a systemic biomarker for HCC, & has been associated with both tumor aggressiveness & response assessment. For measurement of AFP levels, 3 x 10^5 pHCC cells were seeded into 6-well with plates with DMEM+FBS, & allowed to incubate for 72 hours, at which point a 1 mL aliquot was removed & tested for AFP using a porcine AFP ELISA assay kit (MyBioSource, Inc.). Testing was performed in triplicate. Fourteen of 14 (100%) pHCC cell lines expressed AFP, which measured median 12,773 (range 8,631-15,089) ng/dL, mirroring production by HepG2 cells (14,909 ng/dL).

SCID Mouse Xenografts

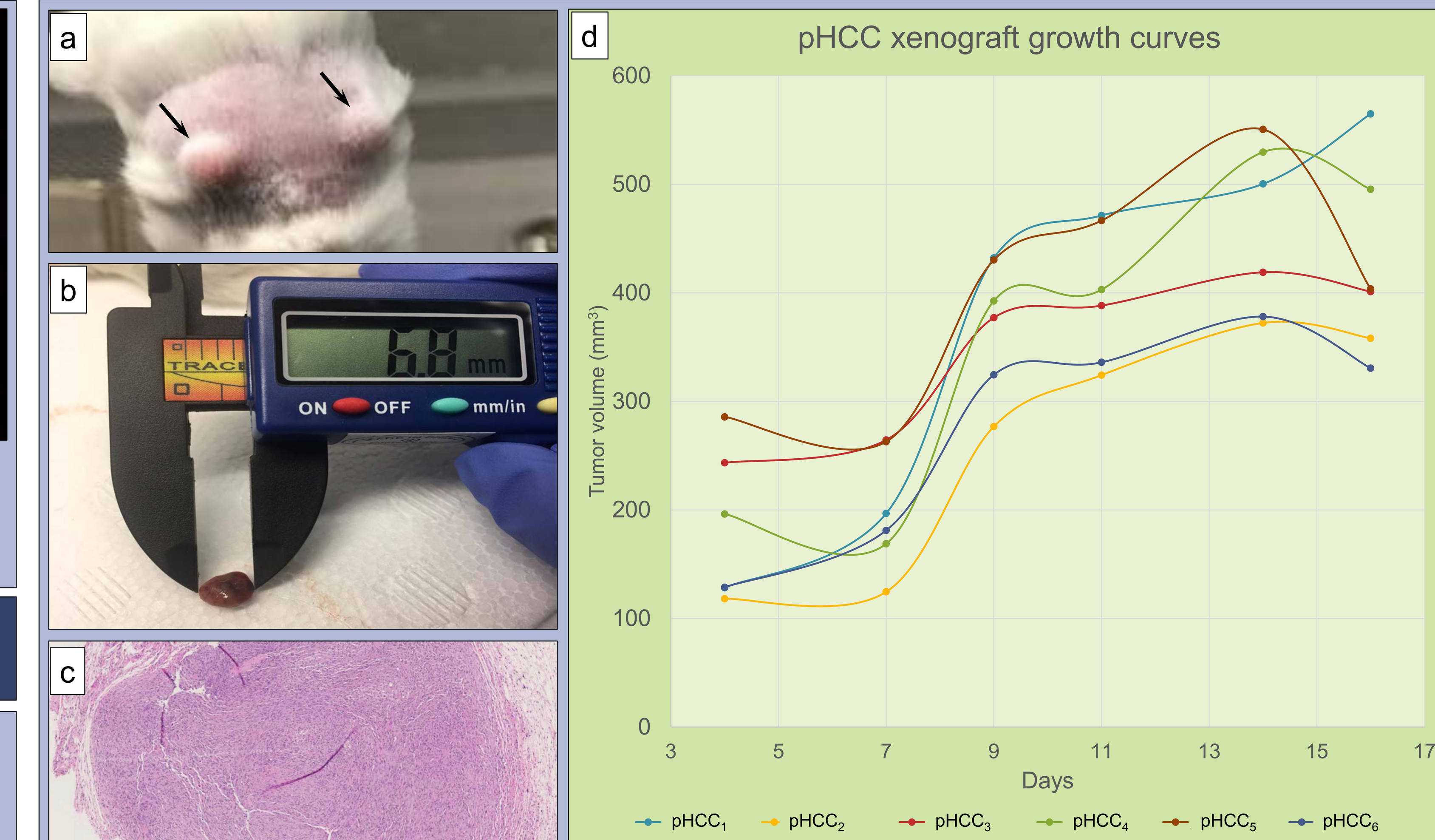


Figure 6. A suspension of 10^7 pHCC cells were inoculated into the SQ tissues of the bilateral flanks of SCID mice (n ≥ 3 per pHCC cell line) to confirm malignant growth. Tumors were measured 3x weekly, & were harvested at 21 days post-injection. SQ tumors were successfully yielded after 76% (74/98) injections, & were median 6.1 x 5.4 mm in size (median volume = 65.4 mm^3, range 4.5-680.7 mm^3). Photograph (a) demonstrates visible tumor masses (arrows) in SCID mouse flank; photograph (b) depicts explanted tumor after animal subject euthanasia & harvest; (c) H & E histologic image reveals neoplastic epithelial cells characterized by variation in cytoplasmic & nuclear size, generally large nuclei with prominent single or multiple nucleoli; (d) pHCC xenograft growth curves for 6 representative pHCC cell lines.

OCM Autografts

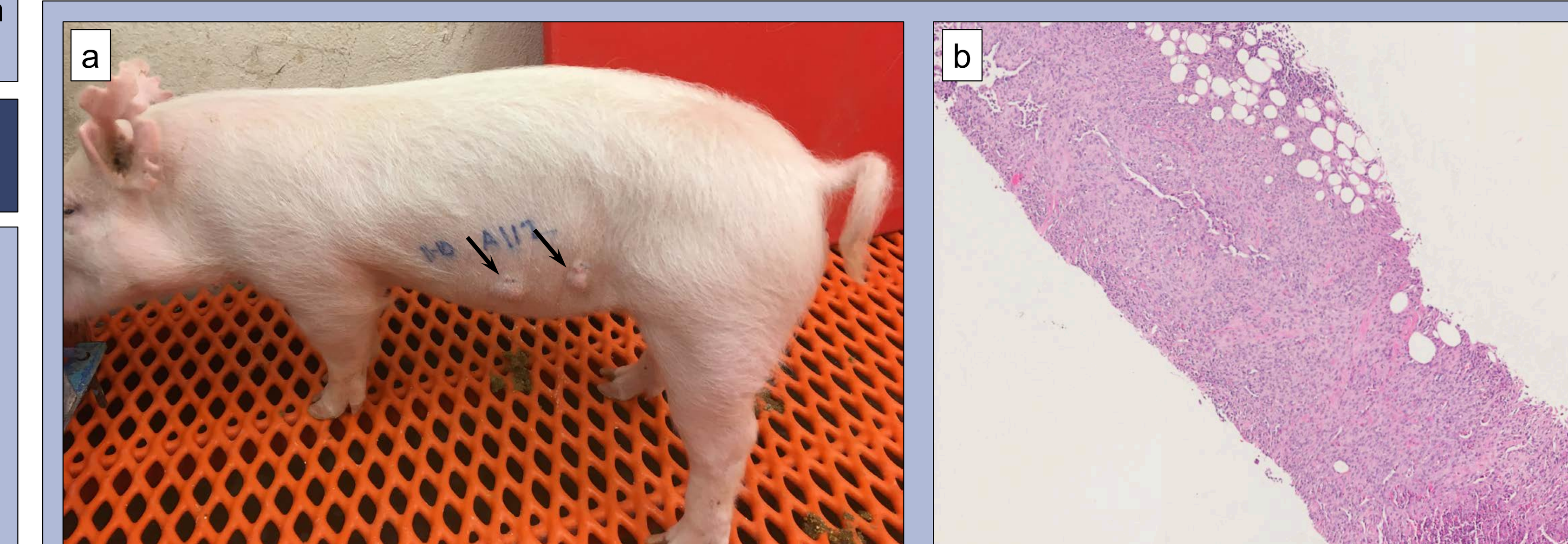


Figure 7. A suspension of 10^7 pHCC cells were inoculated into the SQ tissues of the bilateral flanks of individual donor OCMs (median 3 injections per pHCC cell line) to confirm malignant growth. Tumors were measured 3x weekly, & were biopsied weekly once palpable. SQ tumors were successfully yielded after 63% (27/43) injections, & were median 17.0 x 14.0 mm in size (median volume = 1,628 mm^3, range 80-5,555 mm^3) within 3-43 days post-injection. Photograph (a) demonstrates visible tumor masses (arrows) in OCM flank; H & E histologic image (b) depicts neoplastic epithelial cells characterized by variation in cytoplasmic & nuclear size, generally large nuclei with prominent single or multiple nucleoli; vascularization of the mass & invasion into connective tissue or skeletal muscle also evident.

Conclusions

The results of the current work indicate that pHCC cell lines may be consistently developed from OCMs, & validates OCM pHCC as a platform which accurately replicates human cancer for translational research.

Bibliography

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