Validation of the Oncopig Platform as a Translational Porcine Model for Human Hepatocellular Carcinoma

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Introduction

- Hepatocellular carcinoma (HCC) is the 5th most common cancer globally and 2nd most common cause of cancer death worldwide, with an overall 5-year survival rate of 17.5%.
- A number of unmet clinical needs confront clinicians aiming to improve HCC outcomes.
- A large animal model with genetic, anatomic, and physiologic similarities to humans presenting with relevant comorbidities (i.e. cirrhosis and obesity) is compulsory to transition between preclinical murine models to human clinical trials.
- The Oncopig is a transgenic pig that develops site/cell specific tumors after Cre recombinase induced expression of *KRAS*^{G12D} and *TP53*^{R167H}, mutations found in >50% of human cancers.

Materials and Methods

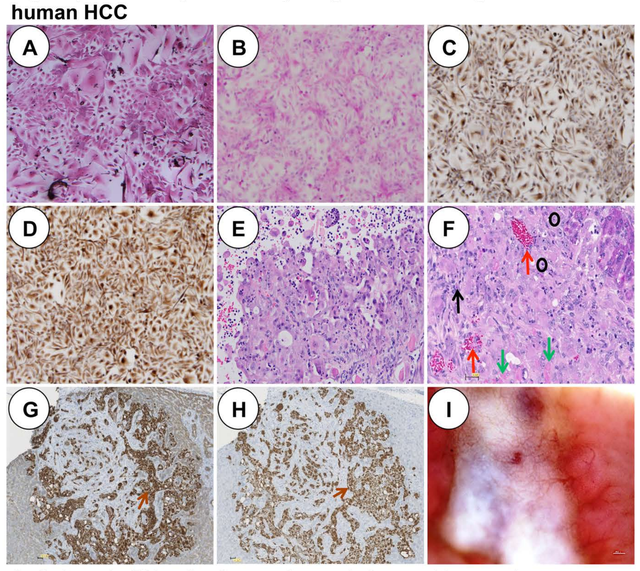
- To validate this HCC model, primary hepatocyte (pPH) cell lines were established from 3 Oncopigs via liver resection and hepatocyte isolation.
- Porcine HCC (pHCC) cell lines were produced from each pPH line via Cre recombinase induced *in vitro* transformation (**Fig. 1**).
- Tumorigenicity was verified by injecting pHCC cells subcutaneously (SQ) and intrahepatically into SCID mice (**Fig. 1**).
- Autologous tumorigenesis was established by injecting pHCC cells into Oncopig SQ sites (**Fig. 2**).
- Genome-wide expression profiles were monitored via RNA-seq (**Fig. 3, 4**), and human HCC cell line expression profiles were extracted from http://medicalgenomics.org/cellminerhcc for comparison of master regulators (**Table 1**).
- Cirrhosis was induced via intravascular administration of an ethanol-Lipiodol emulsion (**Fig. 5**).

Results

Histological Assessment

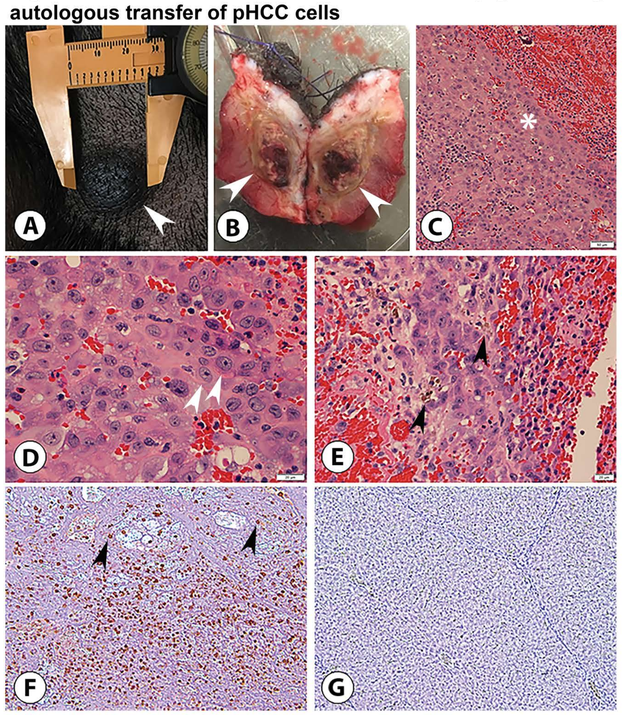
- pPH cells became apoptotic (day 15) and did not produce AFP, while pHCC cells remained viable after 100 passages (confirming malignancy) and secreted AFP (40-50 ng/mL).
- pPH and pHCC cells expressed hepatocyte-specific markers (*ALB*, *HNF4A*, and *G6PC*), while only pHCC cells expressed *KRAS*^{G12D} and *TP53*^{R167H}

Fig. 1. pHCC recapitulates cytologic and histologic features of human HCC



A. Cultured pPH cells show polygonal shape with granular cytoplasm (H&E). **B.** Cultured pleomorphic elongated pHCC cells have clear to granular cytoplasm and round to oval pleomorphic nuclei (H&E). Positive **C.** cytokeratin and **D.** vimentin staining of cultured pHCC cells. **E.** SQ and **F.** intrahepatic xenografted tumors reveal human HCC characteristics including blood vessels (red arrows), stroma (black arrow), neoplastic cells (black circles) and necrotic cells (green arrows) at 21 days post injection (H&E). Positive **G.** cytokeratin and **H.** vimentin staining (brown arrow) of pHCC intrahepatic xenografted tumors. **I.** Blood vessel development in pHCC intrahepatic xenografted tumor.

Fig. 2. Subcutaneous HCC formation in an Oncopig following autologous transfer of pHCC cells

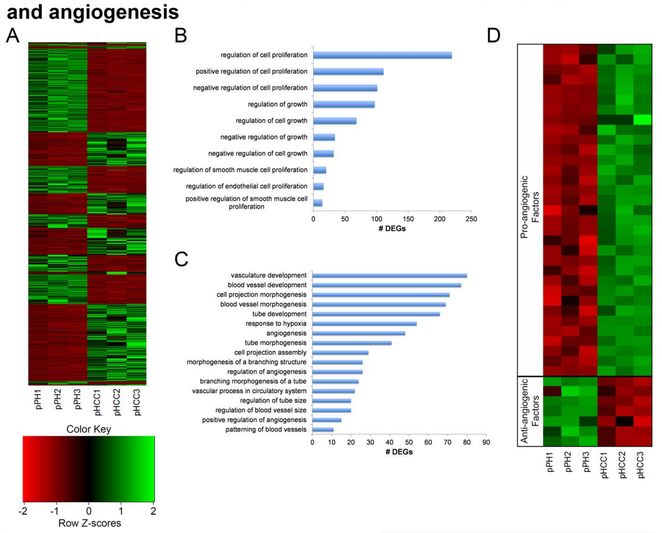


A. 2.7 cm SQ nodule (arrowhead) at 46 days post injection. **B.** Excised nodule displays transected ovoid subcutaneous tumor (arrowheads). **C-E.** H&E examination shows findings consistent with Edmondson-Steiner grade 2 HCC, including a trabecular pattern of malignant cells (* in panel C), characterized by acidophilic and granular cytoplasm, ovoid to round nuclei, and increased nuclear to cytoplasmic ratio (arrowheads in panel D) containing wispy brown material (arrowheads in panel E) representing bile. **F.** Positive CD3 staining reveals diffuse CD3 T-lymphocyte (arrowheads) infiltration into tumor, indicating a "hot" tumor potentially susceptible to immunotherapy. **G.** Negative CD3 staining of control (non-tumorous) Oncopig liver.

Results (cont.)

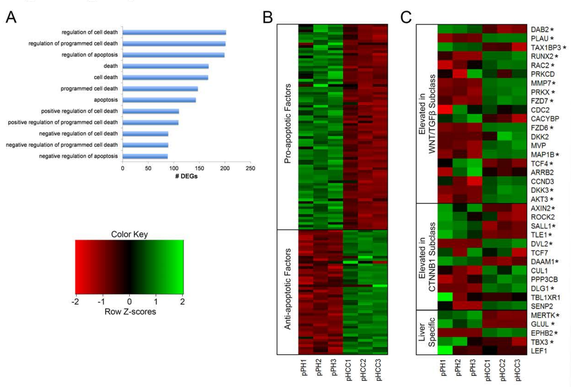
Transcriptional Profiling

Fig. 3. pHCC cell line expression related to cell cycle progression and angiogenesis



A. Heat map of normalized expression levels of 3,481 differentially expressed genes (DEGs) across cell lines. **B.** Enriched gene ontology (GO) terms associated with regulation of cell growth and proliferation. **C.** Enriched GO terms associated with regulation of angiogenesis. **D.** Heatmap of pro- and anti-angiogenic factors displaying elevated and reduced expression in pHCC cell lines, respectively. Expression represented as z-scores.

Fig. 4. Gene expression related to apoptosis and Wnt signaling in pHCC cell lines



A. GO terms enriched for DEGs associated with regulation of apoptosis. **B.** Heatmap of pro- and anti-apoptotic factors displaying reduced and elevated expression in pHCC cell lines, respectively. **C.** Expression profiles of genes whose elevated expression is used to classify human HCC, indicating pHCC cell lines represent the WNT/TGFβ subclass. Expression represented as z-scores. * denotes q < 0.05.

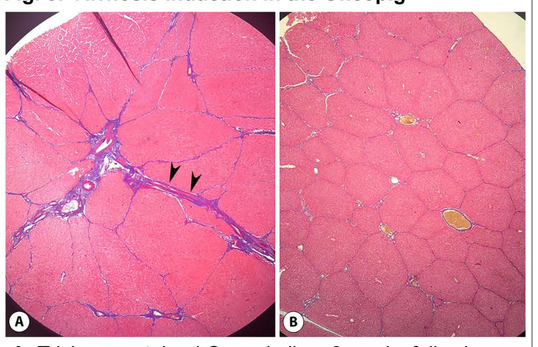
Table 1. Master regulators of genes with reduced expression in Oncopig and human HCC cell lines

Transcription factors	Oncopig pHCC	7703	Focus	Hep3B	Hep3B-TR	Hep40	HepG2	HLE	HLF	HUH-1	HUH-6	HUH-7	SK-Hep1	SNU-182	SNU-387	SNU-389	SNU-449	SNU-475	PLC/PRF/5
STAT1	769	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
EP300	607	431	456	450	438	596	307	545	392	343	379	385	488	270	462	664	652	626	410
FOXA2	541	384	418	394	396	553	298	477	362	312	349	218	451	245	431	638	577	561	386
SPI1	535	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
FOXA1	532	448	478	441	452	630	324	559	408	368	398	382	506	286	506	712	657	667	449
HNF4A	522	435	463	421	429	556	304	542	383	347	387	370	477	285	436	672	654	644	408
HNF4G	391	408	424	421	378	550	293	500	364	331	351	382	458	248	445	628	597	584	390
CEBPB	337	372	384	380	276	357	33	442	339	221	235	252	419	244	408	553	527	535	366
HNF1A	274	–	–	–	–	–	167	–	330	–	–	–	–	–	–	–	–	–	–
NFIC	234	215	226	122	179	169	–	257	208	173	114	172	243	128	228	186	179	291	205
HDAC2	223	353	396	369	206	498	120	451	320	157	147	161	396	236	387	497	442	489	346
NR2F2	174	152	149	137	141	183	95	179	141	123	129	124	170	80	153	218	204	198	148
NR3C1	156	–	–	91	–	–	–	–	–	–	–	157	–	–	–	–	–	–	–
FOXA3	115	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
GATA3	102	–	–	–	–	–	69	–	–	–	–	–	–	–	–	–	–	–	–
E2F1	77	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–	–
STAT2	41	–	–	–	–	–	41	–	–	–	–	–	–	–	–	–	–	–	–

The number of target genes with reduced expression for each transcription factor is indicated for each cell line. (-) Indicates that transcription factor target genes were not enriched (i.e. overrepresented) in the list of genes with reduced expression for a given cell line.

Modeling Comorbidities

Fig. 5: Cirrhosis induction in the Oncopig



A. Trichrome stained Oncopig liver 8 weeks following cirrhosis induction shows irregular hepatic lobules circumferentially surrounded by thick fibrous septa (arrowheads) consistent with stage 4 liver fibrosis (cirrhosis). **B.** Trichrome stain of control liver shows morphologically normal hexagonal hepatic septa.

Conclusions and Future Work

- Oncopig HCC recapitulated key histologic features of human HCC, including an epithelial-mesenchymal transition (EMT), and intracellular pleomorphisms with pale to granular cytoplasm containing single polygonal nuclei *in vitro*.
- Oncopig HCC tumors were histologically characterized as Edmondson Steiner grade 2 HCC with trabecular patterning, pseudoacini patterning, sheets of well-vascularized stroma, EMT, and T-lymphocyte infiltration.
- Human HCC transcriptional hallmarks were detected in pHCC cells, including *TERT* reactivation, apoptosis evasion, angiogenesis activation, altered cell cycle regulation, and Wnt signaling activation.
- Master regulators of gene expression were conserved across Oncopig and 18 human HCC cell lines.
- These results confirm the utility of Oncopig HCC as a clinically relevant model to investigate IR locoregional and targeted therapies of human HCC in translational studies, helping to improve detection, treatment, and biomarker discovery relevant to human HCC.
- Further work is underway to graft SQ autologous tumors into Oncopig livers presenting both with and without cirrhosis.