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The Oncopig cancer model as a validated model for human hepatocellular carcinoma

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Introduction:

Hepatocellular carcinoma (HCC) is the most common form of liver cancer in adults. It is the fifth most common cancer globally and is increasing in incidence in the USA due to high prevalence of comorbidities including hepatitis C and non-alcoholic fatty liver disease as risk factors for HCC development. Current animal models for HCC are severely lacking, and have minimal applicability for translational to clinical practice. Pigs share many genetic and physiological similarities with humans. In a previous study, we created the Oncopig, a transgenic pig encoding Cre recombinase inducible porcine transgenes $Kras^{G12D}$ and $TP53^{R167H}$, a commonly mutated oncogene and tumor suppressor found in over 50% of human cancers, respectively (1). Therefore we investigated the potential of developing a porcine model of human HCC.

Hypothesis:

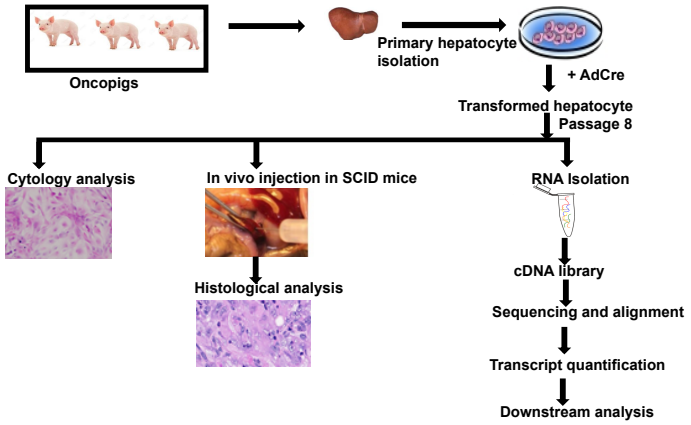
The Oncopig model mimics human HCC providing an ideal translational research platform for improving detection, treatment and other unmet clinical needs for HCC.

Aims:

1. To validate the Oncopig as model for human HCC in terms of phenotype, tumor development and gene expression.

2. To determine the reproducibility of human HCC characteristics across three Oncopigs

Fig. 1: Study design



Materials and methods

- Hepatocytes were isolated from the liver of 3 Oncopigs two of which were from the same litter
- The hepatocytes (pPH) from each Oncopig were transformed by addition of AdCre and injected into 3 SCID mice (a total of 9 mice) at passage 8 and cell number of $1x10^6$ of the transformed cells (pHCC)
- RNA was extracted from primary hepatocytes and transformed hepatocytes of each Oncopig
- The transformed hepatocytes were tested for the expression of $Kras^{G12D}$ and $TP53^{R167H}$ by RT-PCR
- RNA-seq was performed on Illumina HiSeq 2000
- The libraries were sequenced to a total read length of 100 bp from both ends (paired-end sequencing)
- Reads alignment were performed using Tophat2
- Differentially expressed gene(DEG) analysis was performed using Cufflinks

Results:

Fig. 2: pHCC cell lines and intrahepatic tumors recapitulate features of human HCC

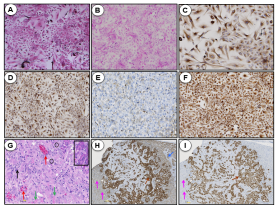


Fig. 3: Transgene and hepatocyte specific gene expression

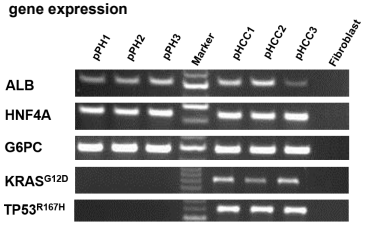


Fig. 4: Hepatocyte genes are downregulated in pHCCs similar to human HCC

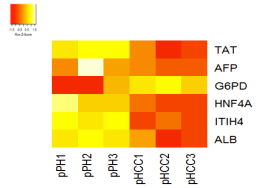


Table1: RNA-seq statistics on Oncopigs

Oncopig	Cell type	Number of reads
1	pPH cells	28,385,664
	pHCC cell line	31,047,086
2	pPH cells	30,286,514
	pHCC cell line	32,296,259
3	pPH cells	23,391,879
	pHCC cell line	29,239,571

Table 2: Number of DEGs in pHCC cell lines

Number of genes upregulated	1811
Number of genes downregulated	1681
Fold change	≥2 or ≤-2
q value	0.05

Fig.5: Gene expression patterns show reproducibility across pPH and pHCC cell lines

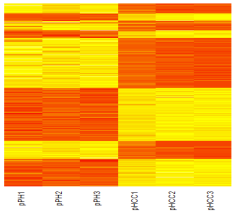
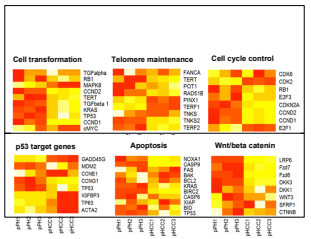


Fig. 6: Oncogenic pathways are dysregulated in pHCC cell lines



Conclusions and implications:

- Cytological analysis revealed that pHCC cells were different from pPH cells and had microscopic similarities with human HCC: As seen in Figure 2: pPH cells were polygonal in shape (A) whereas the pHCC cells were elongated similar to human HCC (B).Both pPH (C) and pHCC cells (D) expressed cytokeratin whereas only pHCC cells expressed vimentin (F). pPH cells (E) did not express vimentin. Expression of both cytokeratin and vimentin is characteristic of human HCC and is indicative of the EMT transition. Histological analysis revealed pHCC cells had morphological (G) similarities with human HCC and also expressed cytokeratin (H) and vimentin (I).
- As observed in human HCC, pHCC cell lines expressed the transgenes $Kras^{G12D}$ and $TP53^{R167H}$ (Fig. 3) and showed a downregulation of hepatocyte genes (Fig. 4).
- DEGs revealed that pHCC cell lines had similar gene expression profile indicating replicability of direction of gene dysregulation in all 3 pHCC cell lines (Fig. 5)
- Oncogenic pathways and genes dysregulated in human HCC were dysregulated in pHCC cell lines (Fig. 6).
- We have established 3 Oncopig hepatocellular carcinoma cell lines which demonstrate reproducibility in recapitulating human HCC in terms of phenotype, tumor development and gene expression. In the future, it is anticipated that the Oncopig will enable us to test different HCC treatment techniques, and evaluate responses both radiographically and pathologically.

References:

1. Schook LB, Collares TV, Hu W, Liang Y, Rodrigues FM, Rund LA, et al. A genetic porcine model of cancer. PLoS One. 2015;10(7), e0128864.

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