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# Porcine drug metabolism and toxicity *in vitro* model utilizing transformed hepatocyte cell lines (pHCC)

**Arun Kumar De[1], Kwame A Darfour-Oduro[1], Laurie Rund[1], Kyle M. Schachtschneider[1,2], Kuldeep Singh[3], Lawrence B Schook[1,4,*]**

[1]Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, Illinois, USA; [2]Animal Breeding and Genomics Center, Wageningen University, Wageningen, The Netherlands; [3]Genzyme-A Sanofi Company, Framingham, MA; [4]University of Illinois Cancer Center, Chicago, Illinois, USA; *Correspondence e-mail: schook@uillinois.edu

## Background

To date, *in vitro* cytotoxicity assays are not highly predictive of *in vivo* toxicity. The adverse effects of new drugs are often not discovered until preclinical animal safety studies or even clinical trials; 40% of drugs drop out in preclinical animal studies and 89% of those that reach clinical trials fail. There is a critical need for more predictive and reliable *in vitro* testing methods. Due to its physiological similarities with humans, pigs have emerged as a suitable and reliable animal model for pharmacological and toxicological studies (Schook et al., 2015a). We developed and characterized a porcine hepatocyte cell line (pHCC) to support drug toxicity and metabolism assessments.

## Objectives

- To develop and characterize a porcine hepatocyte cell line representative of primary hepatocytes to support drug toxicity and metabolism assessments.
- To validate the *in vitro* porcine drug metabolism model in terms of Drug Metabolism Enzyme (DME) gene expression and hepatotoxicity assessment.

## Materials & Methods

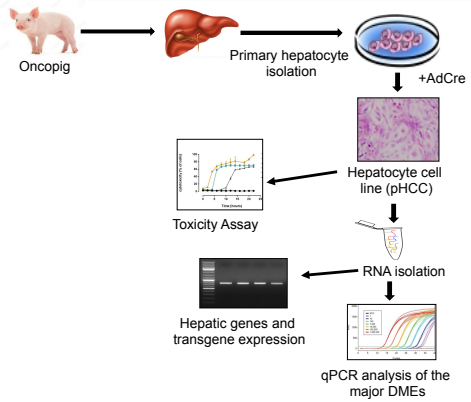


**Fig. 1:** Schematic diagram of the experimental design

- pHCC cells were developed by AdCre activation of Oncopig (Schook et al., 2015b) hepatocytes.
- The expression levels of hepatocyte specific and DME transcripts in pHCC and primary hepatocytes (pPH) were studied.
- The effect of model hepatotoxic compounds (Aflatoxin B1, amiodarone, chlorpromazine and acetaminophen) and selective cytochrome P450 (CYP) modulators (3-methylcholanthrene, rifampicin and phenobarbital) on pHCC cells was evaluated and compared to primary hepatocytes and human models.

## Results

### Porcine hepatocytes are epithelial in origin and have similar morphology to human

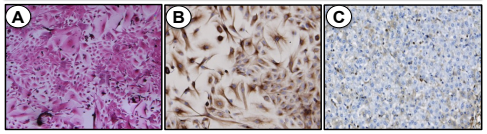


**Fig. 2:** (A) H&E stained porcine primary hepatocytes (B) Expression of cytokeratin in primary hepatocytes. (C) Primary hepatocytes were negative for vimentin.

### Relative abundance of DME genes in porcine primary hepatocytes is consistent with human primary hepatocytes

**Table 1: Relative abundance of DME genes in porcine and human primary hepatocytes**

| Gene | pPH1 | pPH2 | pPH3 | hPH |
|---|---|---|---|---|
| **Phase I DME** | | | | |
| CYP1A1 | 0.00273 | 0.00288 | 0.00312 | 0.0028 |
| CYP1A2 | 0.02187 | 0.02305 | 0.02493 | 0.0173 |
| CYP2A19 | 0.00038 | 0.00043 | 0.00045 | 0.0002 |
| CYP2C33 | 0.05513 | 0.07583 | 0.07053 | 0.0512 |
| CYP2C49 | 0.07076 | 0.08120 | 0.07839 | 0.0754 |
| CYP2E1 | 0.01484 | 0.01153 | 0.01247 | 0.0174 |
| CYP3A | 0.05668 | 0.05942 | 0.04987 | 0.0115-0.0562 |
| CYP7A1 | 0.00034 | 0.00036 | 0.00039 | 0.0001 |
| **Phase II DME** | | | | |
| SULT1A3 | 0.01827 | 0.02032 | 0.02025 | 0.0214 |
| SULT1B1 | 0.03654 | 0.04064 | 0.04051 | 0.0403 |
| SULT2A1 | 0.03457 | 0.03791 | 0.03526 | 0.0442 |
| SULT1E1 | 0.00108 | 0.00118 | 0.00110 | 0.0015 |
| GSTO1 | 0.29733 | 0.29502 | 0.29075 | 0.2539 |
| GSTK1 | 0.14151 | 0.12309 | 0.15677 | 0.1393 |
| **Phase III DME** | | | | |
| ABCB1 | 0.15931 | 0.17591 | 0.16307 | 0.2388 |
| ABCB6 | 0.08133 | 0.14289 | 0.08153 | 0.1141 |
| ABCC2 | 0.38237 | 0.04976 | 0.43937 | 0.4696 |
| ABCC3 | 0.19119 | 0.20419 | 0.19184 | 0.2403 |
| ABCG2 | 0.00216 | 0.00237 | 0.002204 | 0.0061 |

Expression value is a relative number calculated based on the assumption that average expression level of two housekeeping genes GAPDH and ACTB is 1.

Expression values of human primary hepatocytes are reported by Guo et al., 2011

pPH1: Porcine primary hepatocyte 1, pPH2: Porcine primary hepatocyte 2, pPH3: Porcine primary hepatocyte 3, hPH: Human primary hepatocyte

### Primary porcine hepatocytes have limited life span in culture

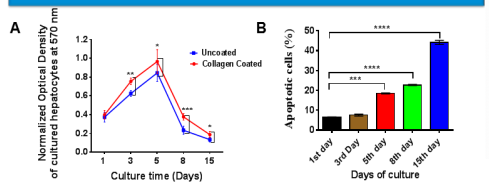


**Fig. 3:** (A) Hepatocyte growth at different days of culture. (B) The histogram shows the mean number of apoptotic hepatocytes (mean ± SD)

### Hepatocyte cell lines (pHCC) are highly proliferative and have unlimited life span in culture

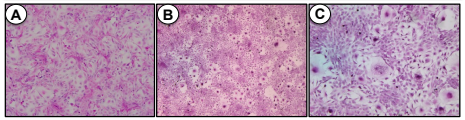


**Fig. 4:** (A) H&E stained pHCC cells (20X) (B) H&E stained pHCC cells cultured in presence of 2% DMSO (10X) (C) H&E stained pHCC cells cultured in presence of 2% DMSO (40X). The cells went through 80 passages.

### pHCC cells express hepatocyte specific genes

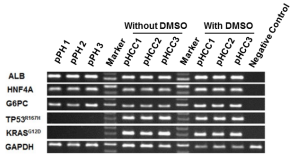


**Fig. 5:** Agarose Gel electrophoresis of RT-PCR products of hepatocyte-specific marker genes; porcine albumin (ALB); HNF4 alpha (HNF4A) and Glucose-6-phosphatase (G6PC). pHCC cells expressed the transgenes ($KRAS^{G12D}$ and $TP53^{R167H}$) while primary hepatocytes did not.

### pHCC cells cultured in presence of DMSO express drug metabolism and regulation genes comparable to primary hepatocytes

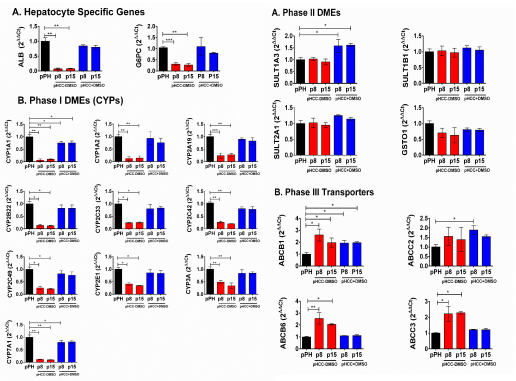


**Fig. 6.** Differential expression profiles of hepatocyte specific and phase I, phase II and phase III DME transcripts in primary hepatocytes and pHCC cell lines.

### Gene regulation by selective CYP modulators in pHCC cells (+DMSO) follows a similar pattern as in primary hepatocytes

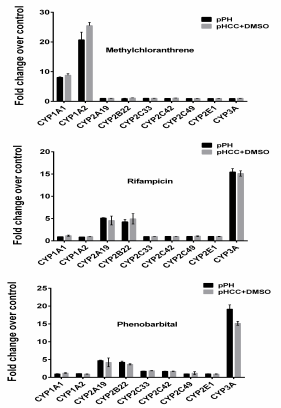


**Fig. 7:** Effect of selective CYP modulators on P450 enzyme transcript expression in primary hepatocytes and pHCC (+DMSO) cell lines.

### pHCC cells (+DMSO) recapitulate toxicity responses of primary hepatocytes

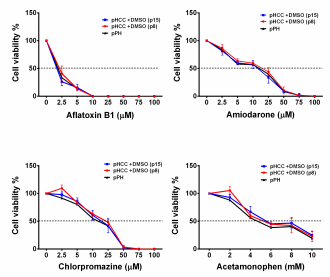


**Fig. 8:** Cytotoxic effects of Aflatoxin B1, amiodarone, chlorpromazine, and acetaminophen on pHCC (+DMSO) cell lines.

## Conclusion

Porcine hepatocyte cell lines (pHCC) represent a useful and predictive *in vitro* model for high throughput screening of new drugs as well as studies on metabolism and hepatotoxicity of chemicals.

## References

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