Metabolic Characterization of Porcine Hepatocytes

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Introduction

In order to be approved for human trials, new drugs need to be tested on mammalian liver cells to determine toxicity. Currently, mouse and rat models are used with limited results because of their vastly different metabolisms. Model systems that utilize primate livers cells are going out of favor because of ethical issues. Cytotheryx Inc. is developing a porcine model system for use in drug trials that are metabolically similar to humans without the ethical concerns. In our research, we developed techniques to characterize porcine liver cell metabolism.[1]

Materials and Methods

Pig hepatocytes that had been treated with DMSO, OMP, RIF, EtOH, and PB were obtained from Cytotheryx, Inc. RNA was isolated using the Roche High Pure RNA Isolation Kit.

Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was used to determine the concentration of RNA in each sample. Applied Biosystems 4x Taq Polymerase master mix was used along with probes and primers obtained from Integrated DNA Technologies.

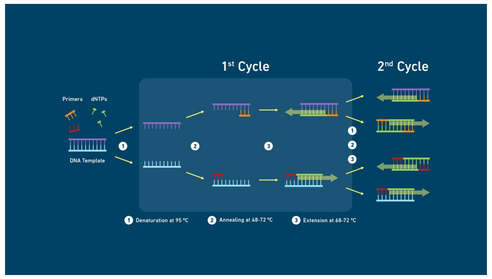


Figure 1. Visual representation of the cycles of qPCR[2].

The Cq values were collected for each reaction and the data was analyzed using the delta-delta Ct method. The RNA expression change for each gene was compared to the correlated solvent in each reaction.

Results

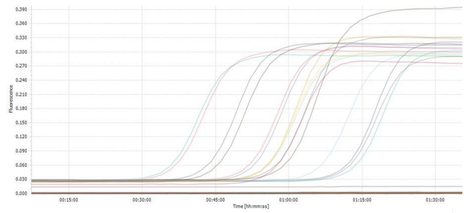


Figure 2. Early qPCR results. Focusing on consistency within triplicates.

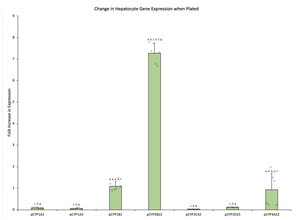


Figure 3. The change in gene expression when cells are plated rather than suspended. The data is significantly different (ANOVA $p < 1.36E-29$). Letters indicate significant differences between the genes in that column. For example, pCYP1A1 is significantly different from columns c, d, and g (pCYP1B1, 2B22, and 3A22) (Turkey HSD: $p < 0.01$).

Conclusions

- The position of cells (plated or suspended) matters for treatment.
- The concentration of treatment impacts gene expression.
- The litter of pig impacts the extent of gene expression, but most genes behave consistently across pigs.
- Gene expression elicits a similar trend from RNA isolation to RNA isolation (cell thaw date) within the same liver sample.
- Gene expression contains a similar trend from liver sample to liver sample.
- Porcine gene expression and in human gene expression under the same treatment conditions elicit similar metabolic trends.

Future Directions

- Key metabolic genes will be further compared to support the use of porcine cells in research, rather than other animals, like mice.
- Analyze human cells transplanted into pigs.
- Improve our qRT-PCR techniques to increase throughput.
- Expand on the genes and treatments being utilized.

Sources

1: (2022, June 29-30). Cellular, Tissue, and Gene Therapies Advisory Committee. https://www.fda.gov/advisory-committees/advisory-committee-calendar/cellular-tissue-and-gene-therapies-advisory-committee-june-29-30-2022-meeting-announcement-06292022.

2: *QPCR analysis, how a qPCR machine works and qPCR protocol*. (2022, January 4). Analysis & Separations from Technology Networks. https://www.technologynetworks.com/analysis/articles/qpcr-analysis-how-a-qpcr-machine-works-and-qpcr-protocol-356835

Results

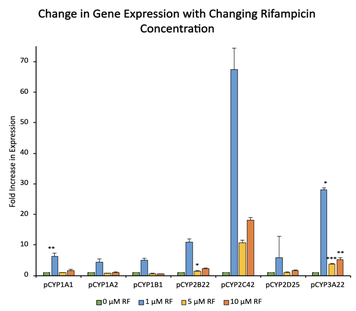


Figure 4. The change in gene expression with Rifampicin concentrations of 0, 1, 5, and 10 µM for July 18 cell samples. * indicates a significant difference of $p < 0.05$ compared to the 0 µM rifampicin concentration. ** indicates a significant difference of $p < 0.01$ and *** indicates a significant difference of $p < 0.001$ compared to the 0 µM rifampicin standard.

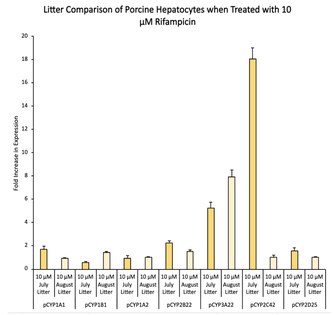
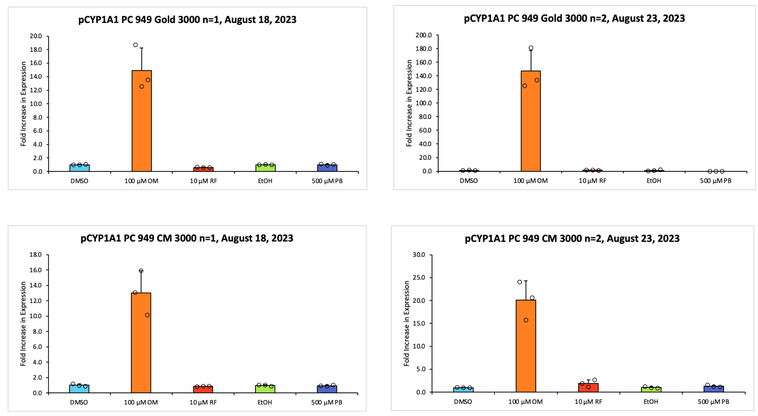
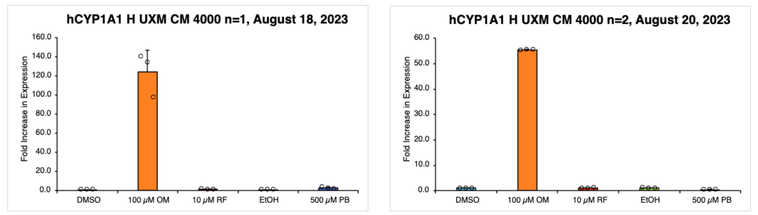


Figure 5. The comparison of hepatocytes treated with 10 µM rifampicin from two different litters of the same porcine species. * indicates a significant difference of $p < 0.05$ between the July litter and the August litter. ** indicates a significant difference of $p < 0.01$ and *** indicates a significant difference of $p < 0.001$ between the July and August litters.



Figures 6-9. The analysis of pCYP1A1 from liver preparations on either August 18, 2023 or August 23, 2023. The cells were cryopreserved, plated in either Gold or CM 3000 media and treated, and then RNA was isolated and protein fold numbers were determined.



Figures 10 and 11. The analysis of hCYP1A1 from liver preparations on either August 18, 2023 or August 20, 2023. The cells were cryopreserved, plated in CM 4000 media and treated, and then RNA was isolated and protein fold numbers were determined.