A BIOMARKER BASED DEVELOPMENTAL TOXICITY SCREEN USING HUMAN INDUCED PLURIPOTENT STEM CELLS FOR COMPOUND PRIORITIZATION

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Introduction

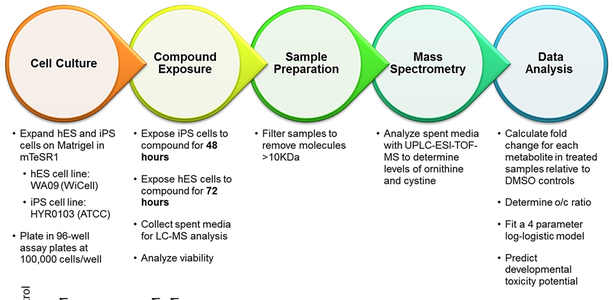
Development of innovative *in vitro* toxicity screening assays aimed at reducing or replacing the use of animal models in compound safety testing is critical to meet the safety requirements for multiple industries. The current initiatives in Europe (Registration, Evaluation, and Authorization of Chemicals, REACH) and the United States (Tox21) to screen thousands of chemicals currently in use for toxicity require inexpensive and innovative *in vitro* models to meet their goals. We have created a predictive developmental toxicity screen that can reduce costs, animal use, and can increase pharmaceutical and chemical safety. A small molecule biomarker-based *in vitro* assay was developed using human induced pluripotent stem (iPS) cells and two metabolites (ornithine and cystine), previously identified as biomarkers of teratogenicity in human embryonic stem (hES) cells (Palmer *et al*., 2013). The assay uses the ratio of the two metabolites (o/c ratio) to indicate the concentration at which a test compound may perturb cellular metabolism in a manner indicative of teratogenicity.

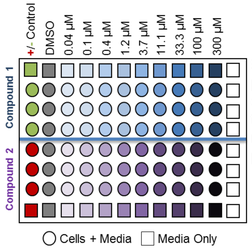
Our present goal was to migrate the assay to an iPS cell-based model by optimizing its design for the cell type. We then tested whether the cells respond to chemical insult in the same reproducible manner as hES cells. iPS cells are derived from the genetic manipulation of human somatic cells and are being widely investigated for use in place of hES cells as a less controversial model. In many ways human iPS cells are phenotypically and genetically similar to hES cells (i.e. morphology, proliferation, gene expression). However, recent research shows that numerous subtle differences exist between the two cell types. To understand what differences might be evident in the biomarker assay we tested 29 compounds (22 training and 7 test set compounds) with known human teratogenicity in both hES and iPS cells. The predictions (teratogen vs. non-teratogen) as well as the concentration at which a compound was predicted to be teratogenic were compared between the two cell lines. Reproducibility of the iPS based assay was tested using at least two replicates of a 10 compound subset. These data show that an iPS cell-based version of the biomarker assay approaches the predictivity of the hES cell-based version (90% concordant). The iPS cell-based assay was highly reproducible with 100% of the replicates showing the same classification and 86% of the teratogenicity potential (TP) values within ± 3-fold of the mean if the TP. The transition of the targeted biomarker assay to iPS cells allows for the option of predictive power approximately equivalent to hES cells without the ethical and political controversy surrounding them.

Methods

Experiment 1: Evaluate the performance of o/c Ratio in iPS cells to predict development toxicants.

Experiment 2: Determine the reproducibility of iPS cell response to treatment.



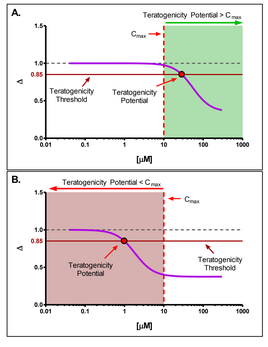


96-well plate layout for test agent evaluation. Each plate contains two test agents (blue and purple colors) treated at 9 concentrations each, a reference treatment (grey) of 0.1% DMSO, a positive and a negative control treatment to track assay performance (red and green, respectively).

Classification scheme applied for known human teratogens and non-teratogens based on therapeutic C_{max} concentration.

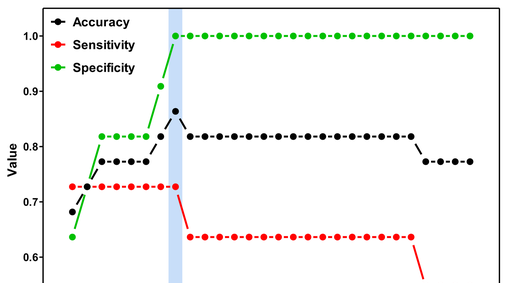
Panel A: A test compound was predicted as a non-teratogen when the teratogenicity potential concentration was higher than the C_{max}.

Panel B: A test compound was predicted as a teratogen when the teratogenicity potential concentration is lower than the C_{max}.



Results

The Teratogenicity Threshold for iPS Cells is Optimal at 0.85



The teratogenicity threshold was adjusted to account for **subtle** differences in response to compound between hES and iPS cells with the optimal value for model accuracy, sensitivity, and specificity determined to be 0.85 (versus 0.88 in hES cells).

Predictions are Similar using hES or iPS Cells

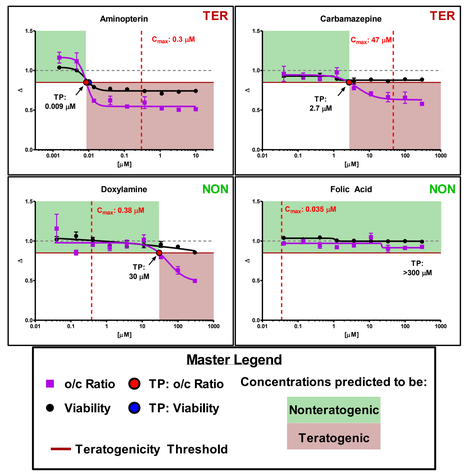
Training Set Results

Compound	Human Effect	Prediction	
		hESC	iPSC
Ascorbic Acid	NON	NON	NON
Caffeine	NON	NON	NON
Diphenhydramine	NON	NON	NON
Doxylamine	NON	NON	NON
Folic Acid	NON	NON	NON
Isoniazid	NON	NON	NON
Levothyroxine	NON	NON	NON
Penicillin G	NON	NON	NON
Retinol	NON	NON	NON
Saccharin	NON	NON	NON
Thiamine	NON	NON	NON
13-*cis* Retinoic Acid	TER	TER	TER
5-Fluorouracil	TER	TER	TER
All-*trans* Retinoic Acid	TER	TER	TER
Busulfan	TER	TER	TER
Carbamazepine	TER	TER	TER
Cytosine Arabinoside	TER	TER	NON
Diphenylhydantoin	TER	NON	NON
Hydroxyurea	TER	TER	TER
Thalidomide	TER	TER	TER
Valproic Acid	TER	TER	TER
Warfarin	TER	TER	NON
	Accuracy	**0.95**	**0.86**
	Sensitivity	0.91	0.73
	Specificity	1.00	1.00

Test Set Results

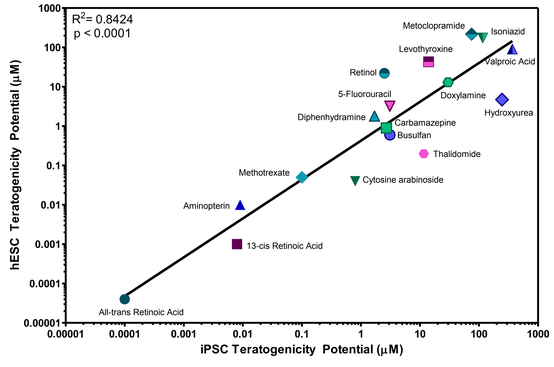
Compound	Human Effect	Prediction	
		hESC	iPSC
Acetaminophen	NON	NON	NON
Acycloguanosine	NON	NON	NON
Amoxicillin	NON	NON	NON
Metoclopramide	NON	NON	NON
Aminopterin	TER	TER	TER
Methotrexate	TER	TER	TER
D-Penicillamine	TER	TER	NON
	Accuracy	**1.00**	**0.86**
	Sensitivity	1.00	0.67
	Specificity	1.00	1.00

Teratogenicity Potential is Dependent on Exposure Level

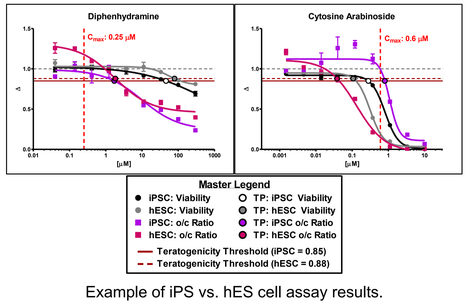


Representative classification of the iPS cell-based targeted biomarker assay results for a subset of compounds.

Teratogenicity Potentials between iPS and hES cells are Similar



Teratogenicity Potential concentrations for compounds that elicit a response in iPS cell (x-axis) and hES cell (y-axis) models were highly correlated (R^2=0.84, p<0.0001).

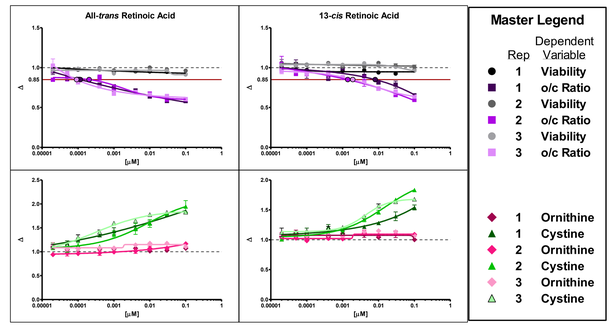


Example of iPS vs. hES cell assay results.

➔iPS and hES cells respond to diphenhydramine at similar concentrations.

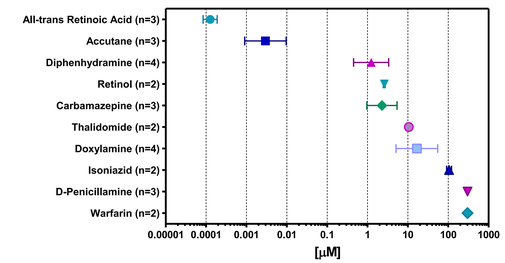
➔hES cells respond to cytosine arabinoside at lower concentrations than iPS cells.

iPS Cell-Based Assay is Highly Reproducible



iPS cell-based assay results across three replicates for each all-*trans* retinoic acid and 13-*cis* retinoic acid were highly reproducible with an average fold change in the TP values across replicates of less than 3-fold. Top Panels show the o/c ratio and cell viability. Bottom panels show individual biomarker response for ornithine and cystine.

Low Variability in the Teratogenicity Potential Across Assay Replicates



Points represent mean ± SD.

Conclusions and Future Directions

- The current study demonstrates that the biomarker-based assay performs well in either an hES or iPS cell-based model.
- Using an optimized teratogenicity threshold of 0.85 for the o/c ratio to account for subtle differences between the cell lines, the iPS cell-based assay had an accuracy of 86% for classifying potential developmental toxicants.
- The hES cell-based assay correctly predicts 28/29 compounds versus 25/29 using iPS cells. The predicted teratogenicity potential concentrations is highly correlated between the two models.
- The iPS cell-based assay is reproducible showing the same classification for all replicates.
- Future development of this assay will focus on exploration of additional biomarkers that may enhance its application.

We recently published the hES cell data used for comparison in *Birth defects research. Part B, Developmental and reproductive toxicology.*

Palmer JA, et al. Establishment and assessment of a new human embryonic stem cell-based biomarker assay for developmental toxicity screening. *Birth Defects Res B Dev Reprod Toxicol*. 2013;**98**(4):343-363.

Acknowledgements

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