DEVELOPMENT OF A TARGETED BIOMARKER ASSAY TO PREDICT DEVELOPMENTAL TOXICITY USING INDUCED PLURIPOTENT STEM CELLS

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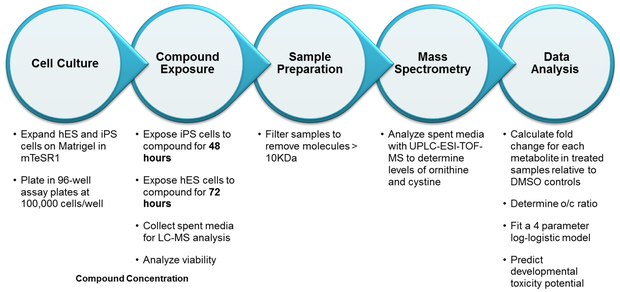
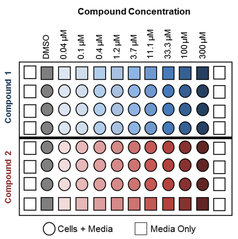
Introduction

Assessment of the developmental toxicity potential of new chemicals is both resource intensive and time consuming. Large numbers of laboratory animals are required and the predictive value of these decades-old tests has been challenged. Availability of more predictive developmental toxicity screens would reduce costs and 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. 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 goal was to determine if the assay could be migrated to an iPS cell-based model by testing whether the cells respond to chemical insult in the same 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. While human iPS cells are phenotypically and genetically similar to hES cells in many respects (i.e. morphology, proliferation, gene expression), recent research has revealed that numerous subtle but important, molecular differences exist. We tested 31 known compounds (23 training and 8 test set compounds) in both hES and iPS cells. The predictions (teratogen vs. non-teratogen) as well as the concentration at which a compound was predicted teratogenic were compared between the two cell lines. The transition of the targeted biomarker assay to iPS cells harnesses the predictive power of the hES cells without the ethical controversy surrounding them.

Methods



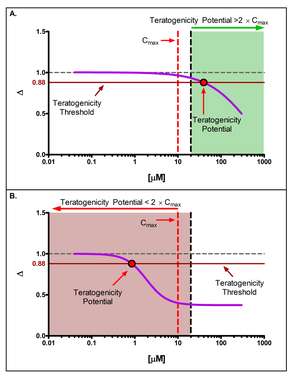


96-well plate layout for test agent evaluation. Each plate contains two test agents (red and blue colors) treated at 9 concentrations each along with a 0.1% DMSO reference treatment (grey).

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 $2 \times C_{max}$.

Panel B: A test compound was predicted as a teratogen when the teratogenicity potential concentration is lower than $2 \times C_{max}$.



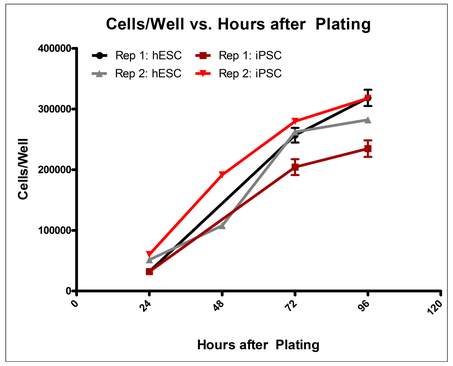
Results

Comparison of hES and iPS Cell Culture Conditions

A set of short experiments were performed to determine if the 96-well assay parameters developed for hES cells would apply to iPS cells.

Question 1: Do iPS cells attach with the same efficiency as hES cells?

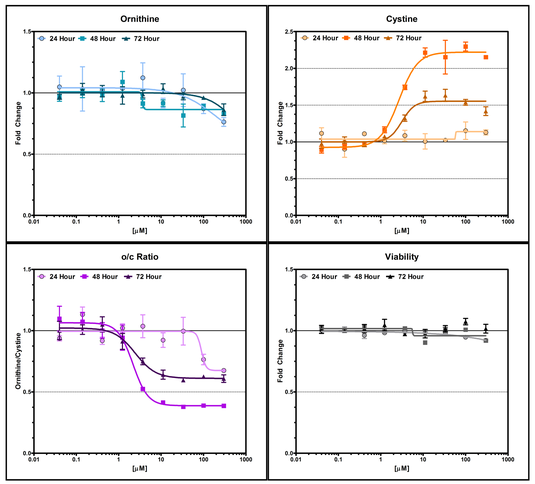
Question 2: Do iPS cells double at the same rate as hES cells in our 96-well culture?



➔Cell number was equivalent between iPS and hES cells 24 hours after plating.

➔Additionally, both cell lines underwent ~3 doublings during the 96 hour culture period.

Question 3: What is the optimum treatment length for iPS cells?



Metabolite response in iPS cells following exposure to carbamazepine.

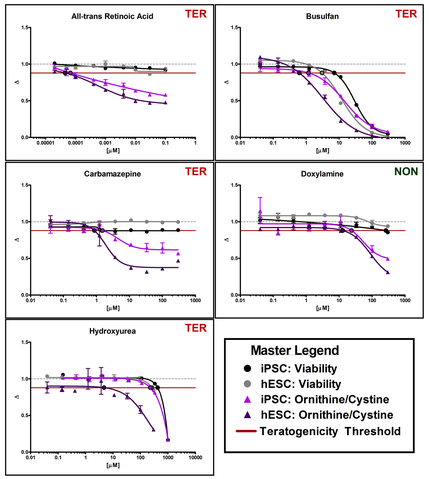
➔Cystine exhibited the greatest response to compound exposure at 48 hours.

➔Ornithine response was more pronounced at 48 hours.

➔Treatment length did not impact the viability response.

➔48 hours was selected as the treatment period in the iPS cell-based developmental toxicity assay.

iPS and hES Cells Exhibit Similar Response to Treatment

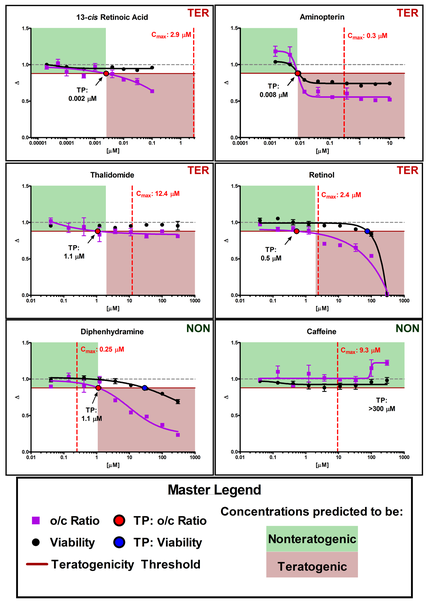


Representative iPS vs. hES cell assay results showing the o/c ratio and cell viability for 4 teratogens and 1 nonteratogen.

➔The interpolated concentration where the o/c ratio crosses the teratogenicity threshold (i.e., Teratogenicity Potential) for all-*trans* retinoic acid, busulfan, carbamazepine and doxylamine is within 2-fold.

➔Hydroxyurea is an example of a compound where the o/c response differs between the two cell types.

Developmental Toxicity Classification



Representative classification of iPSC assay results for a subset of the test compounds.

Teratogenicity Prediction based on Therapeutic C_{max}

Compound	Human Effect	hES Cells		iPS Cells	
		Teratogenicity Potential (µM)	Prediction	Teratogenicity Potential (µM)	Prediction
Ascorbic Acid	NON	>300	NON	>300	NON
Caffeine	NON	>300	NON	>300	NON
Diphenhydramine	NON	1.8	NON	1.1	NON
Doxylamine	NON	13	NON	24	NON
Folic Acid	NON	>300	NON	>300	NON
Isoniazid	NON	173	NON	43	TER
Levothyroxine	NON	43	NON	10	NON
Penicillin G	NON	>300	NON	>300	NON
Retinol	NON	22	NON	0.5	TER
Saccharin	NON	>300	NON	>300	NON
Thiamine	NON	>300	NON	>300	NON
B1: Acetaminophen	NON	278	NON	>300	NON
B2: Acycloguanosine	NON	108	NON	179	NON
B3: Amoxicillin	NON	>300	NON	>300	NON
B4: Metoclopramide	NON	218	NON	69	NON
13-*cis* Retinoic Acid	TER	0.001	TER	0.002	TER
5-Fluorouracil	TER	3.2	TER	3.5	TER
All-*trans* Retinoic Acid	TER	0.00004	TER	0.00007	TER
Busulfan	TER	0.6	TER	1.2	TER
Carbamazepine	TER	0.9	TER	1.5	TER
Cytosine arabinoside	TER	0.04	TER	0.8	TER
Diphenylhydantoin	**TER**	165	**NON**	291	**NON**
Hydroxyurea	TER	4.7	TER	227	TER
Methotrexate	TER	0.05	TER	0.08	TER
Thalidomide	TER	0.2	TER	1.1	TER
Valproic Acid	TER	91	TER	321	TER
Warfarin	TER	6.5	TER	>300	NON
B5: Acrolein	**TER**	272	**NON**	241	**NON**
B6: Aminopterin	TER	0.01	TER	0.008	TER
B7: Flusilazole	TER	<0.04	TER	33	TER
B8: D-Penicillamine	TER	<0.04	TER	>300	NON
	Accuracy	**0.94**		**0.81**	
	Sensitivity	0.88		0.75	
	Specificity	1.00		0.87	

Note: Red text indicates compounds correctly predicted in hESC, but incorrectly predicted in iPSC. **Bolded** compounds were incorrectly predicted in both hESC and iPSC.

Conclusions and Future Directions

- The current study shows proof of concept that the assay can be transferred from an hES cell-based model to iPS cells.
- 27 of the 31 compounds have the same prediction in iPS and hES cell assays.
- Using a teratogenicity threshold of 0.88 for the o/c ratio (determined with hES cell data), the iPS cell-based assay was had an accuracy of 81% for classifying potential developmental toxicants.
- Ongoing research will further define the teratogenicity threshold in the iPS cell-based assay to account for subtle differences in response between the two cell lines.

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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