ESTABLISHMENT AND ASSESSMENT OF A NEW HUMAN EMBRYONIC STEM CELL BASED BIOMARKER ASSAY FOR DEVELOPMENTAL TOXICITY SCREENING

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Introduction

Application of more predictive developmental toxicity screens would aid in reducing the prevalence of birth defects and increase pharmaceutical and chemical safety worldwide. Human embryonic stem (hES) cell technology provides an opportunity for innovative and robust alternative *in vitro* model systems. Previously, we used high resolution mass spectrometry (HRMS) based metabolomics to discover and test two biomarkers indicative of a metabolic perturbation detected in the culture media that could be used as an early signal for potential developmental toxicity. Here these biomarkers (ornithine and cystine) provide the foundation for the transition to a rapid exposure-based *in vitro* teratogenicity assay using hES cells. The teratogenic potential of a compound is associated with the level of exposure to the fetus and this approach takes into account the issue of compound exposure in predicting teratogenicity.

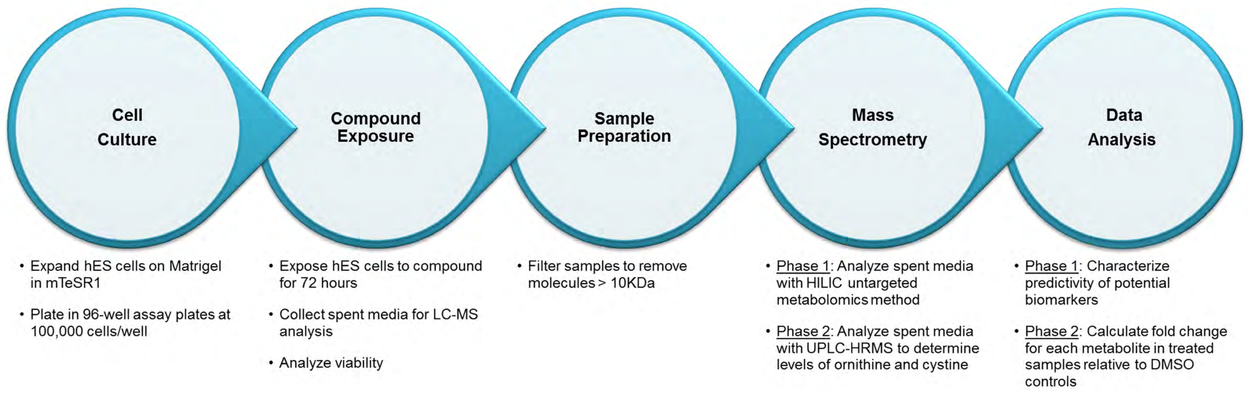
First, hES cells were treated with 23 pharmaceuticals of known human teratogenicity at concentrations relevant to published plasma *in vivo* concentrations. The ratio of two metabolite biomarkers (ornithine and cystine, the o/c ratio) was evaluated as an indicator of developmental toxicity. Next, an exposure-based biomarker assay using the ratio, along with a cytotoxicity endpoint, was used to develop a paradigm to predict developmental toxicity. The predictivity of the new assay was evaluated using the training set and a 13 compound test set. To illustrate its real-world application, the assay was then applied to 10 additional compounds that are not well defined in humans. This assay had a high concordance with existing *in vivo* models, and can predict the developmental toxicity potential of new compounds as part of discovery phase testing and provide insight into the likely outcome of required *in vivo* tests.

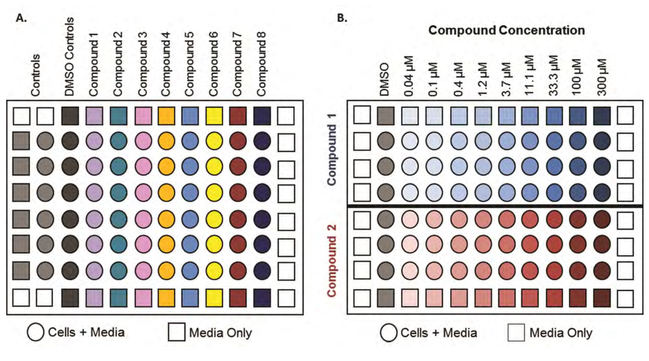
Methods

Development and evaluation of the new assay was conducted in two phases:

Phase 1: The predictive potential of ornithine and cystine was characterized across nine independent experimental replications of the training set using untargeted metabolomic methods.

Phase 2: The predictive biomarkers were used to develop a rapid turnaround, targeted, exposure-based assay for compound prioritization based on teratogenicity potential. The predictivity of the new assay was evaluated using the original training set as well as an independent test set of compounds.

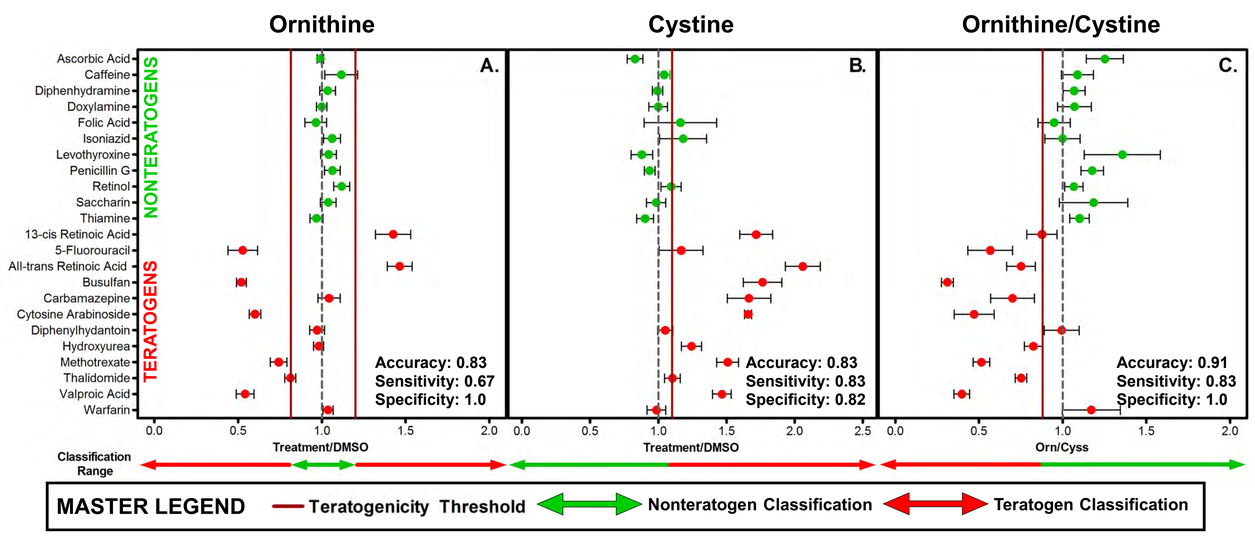




96-well plate layout for test agent evaluation.
(A) Phase 1 untargeted metabolomics
(B) Phase 2 targeted biomarker experiments

Results: Phase 1

A Ratio of Ornithine/Cystine Provides Maximal Predictivity

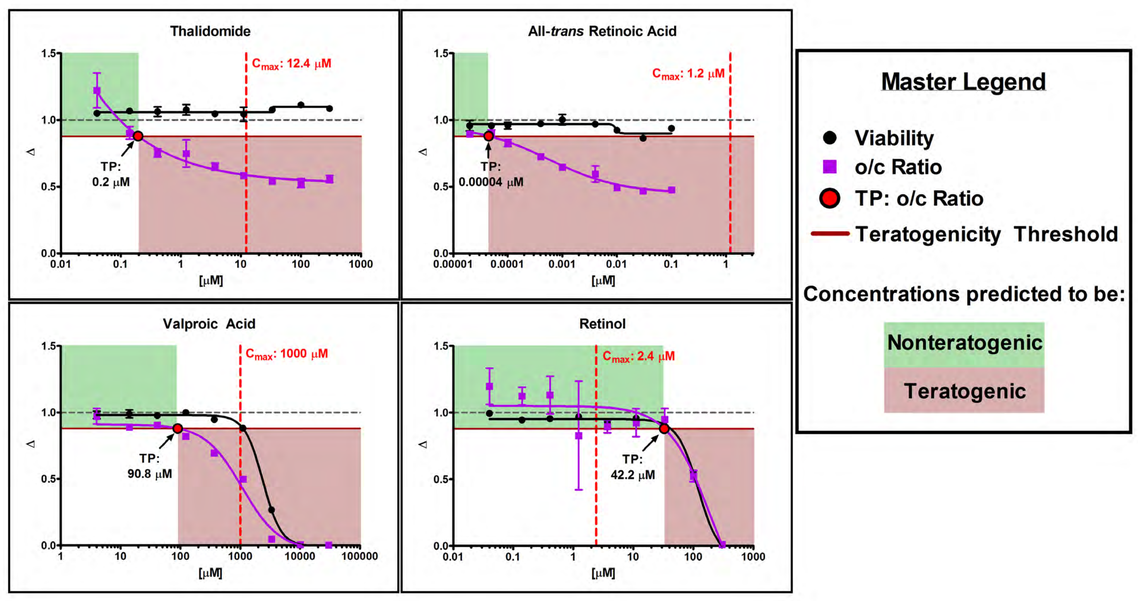


➔Individual metabolites are able to classify potential developmental toxicants with high accuracy.

➔Accuracy was increased when the metabolites are used as a ratio.

Results: Phase 2

Developmental Toxicity Potential is Dependent on Exposure Level



Targeted biomarker assay results for a representative subset of the training set compounds.

➔The metabolites in the o/c ratio respond to compound exposure in the absence of or well before changes in cell viability.

➔The exposure level likely to be encountered by a fetus are critical to consider when making prediction. For example, retinol causes cell death and a change in the o/c ratio. However, these changes occur at concentrations ~20× human circulating levels.

The o/c Ratio is More Accurate and Sensitive than Cell Viability

Assay	Accuracy	Sensitivity	Specificity
Training Set			
o/c Ratio	0.96	0.92	1.00
Cell Viability	0.70	0.42	1.00
Test Set			
o/c Ratio	0.77	0.57	1.00
Cell Viability	0.62	0.29	1.00

Note: Training Set: 23 compounds, 11 nonteratogens, 12 teratogens
Test Set: 13 compounds, 6 nonteratogens, 7 teratogens

Assay Results are Concordant with Published *in vivo* Results

Compound	Teratogenicity Potential (µM)		Rodent *in vivo* test results	
	o/c Ratio	Cell Viability	Teratogenic	Embryotoxic
6-Aminonicotinamide	<0.04	24.5	+	-
Abacavir	95.1	94.1	+	+
Adefovir dipivoxil	0.0015	0.02	-	-
Amprenavir	236.9	259.5	+	+
Artesunate	0.64	0.58	+	+
Cidofovir	0.3	1.9	-	-
Entacapone	6.7	127	+	-
Fluoxetine	25.1	23	-	+
Ramelteon	34	>300	-	-
Rosiglitazone	18.9	21.8	-	+

Conclusions and Future Directions

◈ Metabolites identified using an “omics” based computational model approach captured a biochemical phenotype that was able to discriminate developmental toxicants based on known physiological mechanisms.

◈ Combining 9 exposure levels with cell viability and the o/c ratio provides an opportunity to identify the exposure level at which a test agent alters hES cell growth and metabolism such that it is correlated with teratogenicity potential.

◈ Results from the new assay are highly concordant with published *in vivo* results.

◈ Studies are underway comparing the response to compound exposure between hES and iPS cells using the o/c ratio.

This is a subset of the data from our recent publication:

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.

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