Evaluation of the hPSC Scorecard Assay in a Human Embryonic Stem Cell Test for Developmental Toxicity Screening

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Introduction

The US Environmental Protection Agency (EPA) Center for Computational Toxicology and Exposure employs high-throughput screening approaches to identify environmental chemicals that can pose a risk to human health. Key provisions in the Frank R. Lautenberg Chemical Safety for the 21st Century Act promotes the use of non-animal, new approach methods to identify chemical risks to susceptible populations including pregnant women. Most of the current assays within the US EPA's ToxCast and Tox21 portfolio are not designed to evaluate cellular processes associated with human development, therefore cell-based models that recapitulate signaling pathways for defined endpoints in early embryonic patterning are needed to identify potential hazards during pregnancy. Human induced pluripotent stem cells (hiPSCs) aggregated into 3D embryoid body (EB) cultures have the capacity to spontaneously differentiate into ectoderm, mesoderm and endoderm lineages. This differentiation patterning can recapitulate early embryonic pathways and has been utilized in methods such as the mouse embryonic stem cell test (mEST). While the mEST and other methods have displayed predictive power in identifying > 70% developmental toxicants, they are not capable of identifying toxicants in all areas of development and have yet to be incorporated into regulatory decision making. New models that can measure changes to complex differentiation patterning of a human embryo are needed to advance developmental toxicology testing from current animal model standards.

Objective

To adapt hiPSCs to a 96-well EB stem cell test to identify chemicals that perturb early germ layer differentiation patterning.

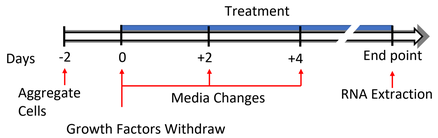
Material and Methods

EBs were formed from 2000 human iPSCs (Gibco) and allowed to aggregate in V-bottom 96-well ultra low adherence plates (S-bio, Hudson NH USA) for 2 days.

At day 0, FGF2 and TGFß were withdrawn and medium with or without chemical were added every two days with a CyBio FeliX automated liquid handler (Analytik Jena, Beverly MA USA).

At end point, mRNA was extracted from 4 pooled EBs using a RNeasy Mini kit (Qiagen, Germantown MD USA).

Embryoid Body Test Time Frame



The commercial TaqMan hPSC Scorecard Assay gene-signature array (Life Technologies, Carlsbad CA USA) was used to conduct temporal analysis of spontaneous differentiation and measure perturbations to differentiation.

The hPSC Scorecard assay is based on work that evaluated 23 hES and hiPSC lines and determined a gene set that can be used to assess stem cell pluripotency and capability to differentiate to germ layer lineages (1).

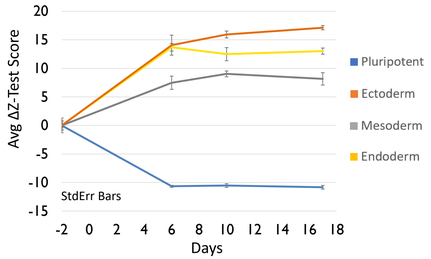
Results and Conclusions

hiPSC Embryoid Body Germ Layer Signature Gene Expression

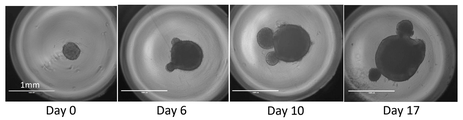
Germ lineage commitment and progression was determined by calculating the average difference in weighted Z-test scores (ΔZ-test) relative to pre-aggregated cells across three separate experiments.

Day 6 was deemed adequate time for all three germ layer profiles to be significantly expressed.

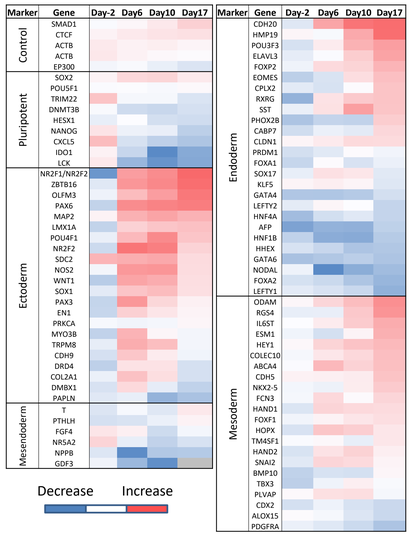
Embryoid Body Germ Layer Differentiation



Embryoid Body Development in Ultra-Low Adherence Well

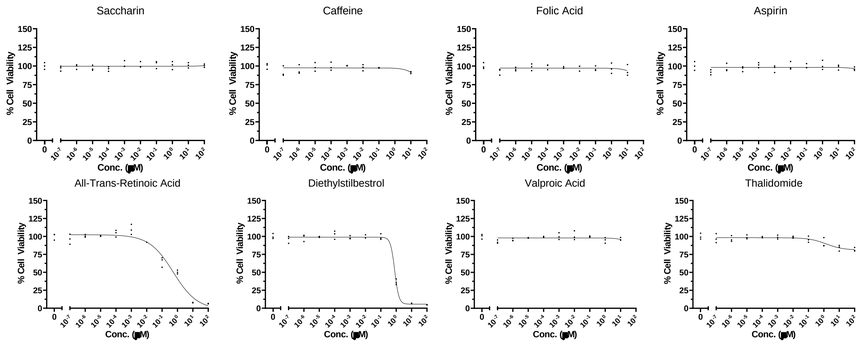


Embryoid Body Gene Expression



Embryoid Body Cell Viability Dosage Test with Reference Chemicals

Concentration range-finding experiments using CellTiter Glo 2.0 were used to define a reference set of eight chemicals ranging the spectrum of FDA pregnancy risk categories (A-X) at known teratogenic and non-teratogenic doses.



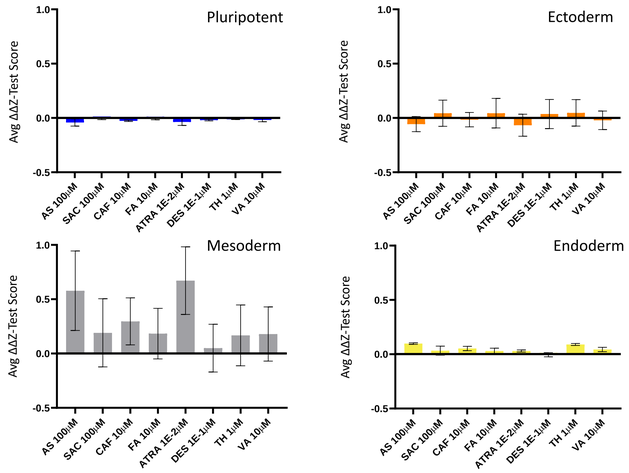
Reference Chemicals and Doses

Reference Chemical	CASRN	Tested Dose (μM)	Teratogenic	Preg. Class
All-Trans-Retinoic acid	302-79-4	0.01	Teratogen	X
Diethylstilbestrol	56-53-1	0.1	Teratogen	X
Thalidomide	50-35-1	1	Teratogen	X
Valproic acid	127-01-1	10	Teratogen	D
Aspirin	50-78-2	100	Non-Teratogen	C
Caffeine	58-08-2	10	Non-Teratogen	B
Folic acid	59-30-3	10	Non-Teratogen	A
Saccharin	81-07-2	100	Non-Teratogen	A

No Significant Change in Embryoid Body Germ Layer Gene Expression with Chemical Exposure

No consistent differences in EB differentiation were observed at day 6 when compared to mean ΔZ-test values for ectoderm (15.6 ± 2.7), mesoderm (5.1 ± 1.6) and endoderm (12.6 ± 0.9) in solvent treated controls.

Embryoid Body Germ Layer Differentiation



Further investigation is warranted to determine if exposure frequency, duration, and endpoint analysis are suitable for evaluating hiPSC EB differentiation using the TaqMan hPSC Scorecard Assay gene panel.

Reference

1) Tsankov AM, Akopian V, Pop R, Chetty S, Gifford CA, Daheron L, et al. A qPCR ScoreCard quantifies the differentiation potential of human pluripotent stem cells. Nature Biotechnology. 2015;33:1182.

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