Evaluation of the hPSC Scorecard Assay in a Human iPSC Embryoid Body Test for Developmental Toxicity Screening

John Gamble[1,2], Chad Deisenroth[1]

[1]Center for Computational Toxicology and Exposure, US EPA Research Triangle Park, NC, USA

[2]Oak Ridge Institute for Science and Education, Oak Ridge, TN, USA

www.epa.gov

Abstract Number: 3035

John Gamble | gamble.john@epa.gov | 919-541-4688

Abstract

The US EPA 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. The objective of this study was to adapt human induced pluripotent stem cells (hiPSCs) to a 96-well embryoid body (EB) stem cell test to identify chemicals that perturb early germ layer differentiation. EB formation, maintenance, and treatment was optimized for seeding density, plate type, and media changes using automated liquid handling. The commercial TaqMan hPSC Scorecard Assay gene-signature array was used to conduct temporal analysis of spontaneous differentiation. Pronounced EB differentiation was noted as early as day six, with maximum germ layer expression plateauing at day ten. Solvent tolerance and concentration-range finding experiments were used to define a reference set of eight chemicals (all-trans retinoic acid, aspirin, caffeine, diethylstibestrol, folic acid, saccharin, thalidomide and valproic acid) ranging the spectrum of FDA pregnancy risk categories (A-X) at known teratogenic and non-teratogenic doses. Disruption of germ lineage commitment and progression was determined after ten days of chemical exposure by calculating the Z-test scores. Solvent treated controls displayed successful EB differentiation with Z-test values for ectoderm (16.3 ± 1.2), mesoderm (4.2 ± 0.7) and endoderm (9.4 ± 2.6). All-trans retinoic acid (0.01µM) and valproic acid (100µM) treatment both provided a difference in EB differentiation with increased mesoderm (8.0 ± 0.5; 5.2 ± 0.4) while all-trans retinoic acid also greatly increased endoderm (23.5 ± 2.1) expression. 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. The views expressed are those of the author and do not necessarily reflect the views or policies of the US EPA.

Objective

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

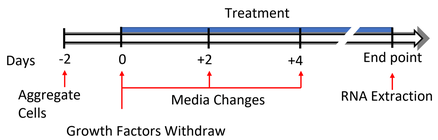
Material and Methods

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.

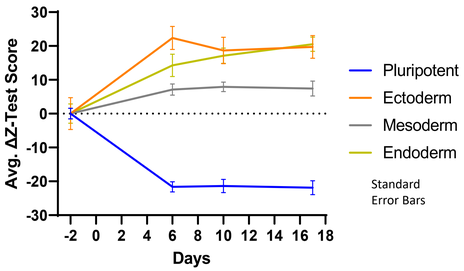
The hPSC Scorecard assay is based on work that evaluated 23 pluripotent stem cell lines and determined a gene set that can be used to assess stem cell pluripotency and capability to differentiate to germ layer lineages (1).

Results and Conclusions

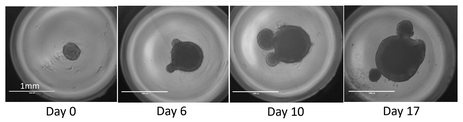
hiPSC Embryoid Body Germ Layer Signature Gene Expression

Germ lineage commitment and progression was determined by calculating the average difference in Z-test scores (ΔZ-test) relative to pre-aggregated cells across three separate experiments. All three germ layer profiles were significantly expressed by day 6 with expression plateauing at day 10.

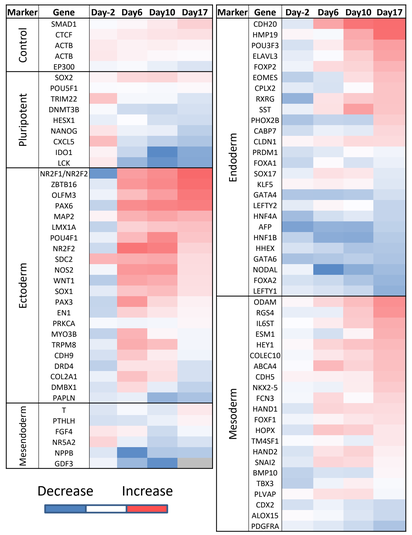
Embryoid Body Germ Layer Differentiation



Embryoid Body Development in Ultra-Low Adherence Well

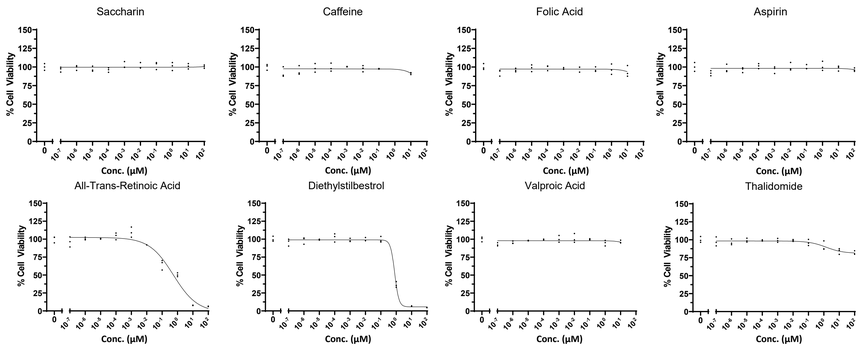


Embryoid Body Gene Expression



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.



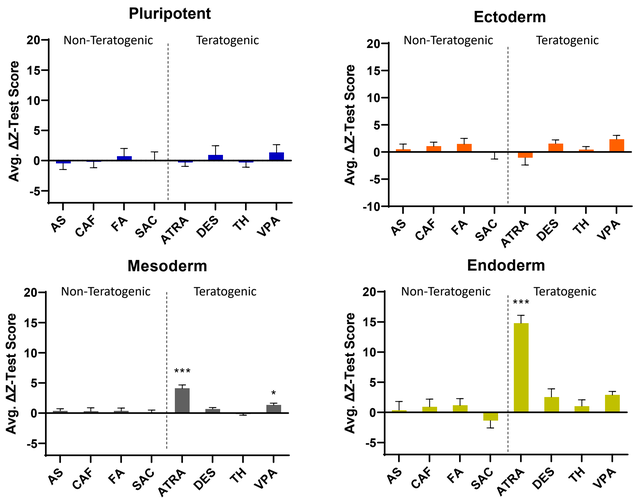
Reference Chemicals and Doses

Reference Chemical	CASRN	Day 10 Dose Tested (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	100	Teratogen	X
Valproic acid	127-01-1	100	Teratogen	D
Aspirin	50-78-2	100	Non-Teratogen	C
Caffeine	58-08-2	100	Non-Teratogen	B
Folic acid	59-30-3	100	Non-Teratogen	A
Saccharin	81-07-2	100	Non-Teratogen	A

Significant Change in Germ Layer Gene Expression With ATRA Exposure

At day 10, 0.01µM ATRA and 100µM VPA significantly increased expression in mesoderm and with ATRA also affecting endoderm associated genes when compared to mean Z-test values for ectoderm (16.3 ± 1.2), mesoderm (4.8 ± 0.7) and endoderm (9.4 ± 2.6) in solvent treated controls.

Embryoid Body Germ Layer Differentiation



ANOVA: P < 0.0001; Dunnett's Multi Comparison Test Against DMSO: ***P < 0.0001, *P < 0.05; Standard Error Bars

The assay correctly identified 50% of the teratogenic chemicals without any false positives. This preliminary data reveals that further investigation is warranted. Evaluation of additional chemicals are needed 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.

U.S. Environmental Protection Agency
Office of Research and Development