Adaptation of a Human Pluripotent Stem Cell Assay for Developmental Toxicity Screening

John Gamble[1,2], Kristen Hopperstad[1], 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

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

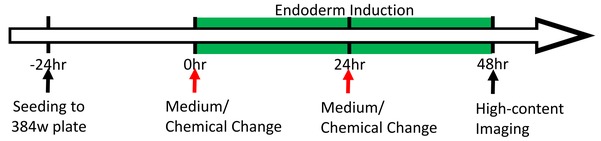
www.epa.gov

Introduction

The US Environmental Protection Agency (EPA) is committed to developing non-animal, new approach methods to detect chemical risks to susceptible populations, including pregnant women. Additional coverage for cellular processes associated with human development in the ToxCast and Tox21 assay portfolio could enhance identification and prioritization of potential developmental toxicants. Human pluripotent stem cells (hPSCs) have the capability to differentiate into any cell type and provide an excellent model to study human development as they recapitulate embryonic differentiation pathways. With the proper signaling, hPSCs can be differentiated into each of the three gastrulation lineages in human development: ectoderm, mesoderm and endoderm. Endoderm differentiation is particularly of interest as it develops into most of the body's organs including the thyroid, lungs, liver and digestive tract. Additionally, endoderm differentiation can be quickly and efficiently produced with hPSCs, making the model amenable to high-throughput screening applications. The Human Pluripotent Stem Cell Test (hPST) utilizes stem cell differentiation towards endoderm to identify teratogens that perturb Sox17 expression, a key endodermal biomarker (1). It has shown great promise in detecting teratogens (94% balanced accuracy) from a limited number of drug-like compounds and other kinase inhibitors. **To adopt the principles of the hPST, we utilized the RUES2-GLR germ line reporter hPSC cell line engineered with fluorescent reporters for biomarkers representing all three gastrulation lineages. The RUES2-GLR assay enables rapid 384-well high-throughput screening and quantitative analysis of multi-lineage differentiation, demonstrating potential to identify and prioritize teratogens based on affected gastrulation lineages.**

Material and Methods

- For this assay, a transgenic human pluripotent stem cell line (RUES2-GLR) containing three fluorescent germ layer reporter biomarkers, Sox2–mCitrine (ectoderm/ pluripotency), Brachyury (Bra)–mCerulean (mesoderm), and Sox17–tdTomato (endoderm), was used to assess perturbations during endoderm differentiation with the PSC Definitive Endoderm Induction kit from Gibco (2).



- After 48 hrs of chemical exposure, cytotoxicity was measured via cell count with nuclear stain, HCS NuclearMask Deep Red Stain (Invitrogen), and the percentage of Sox17+ cells was obtained to examine developmental toxicity using high-content image analysis.

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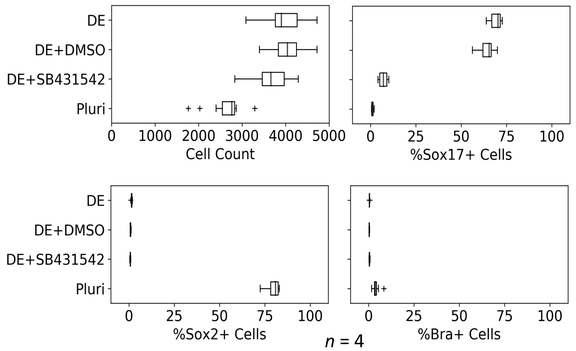
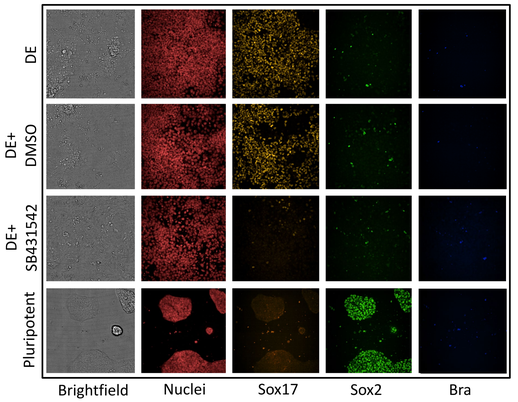
Results and Conclusions

Definitive Endoderm Induction with RUES2-GLR Cells

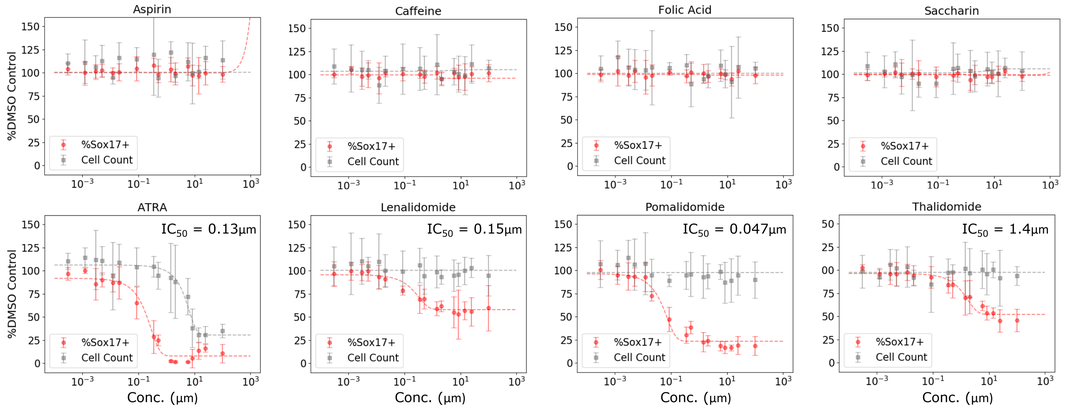
- Pluripotent RUES2-GLR cells had a 79.7% Sox2+ population with a minimal Sox17+ population.
- Directed endoderm induction (DE) resulted in a 68.9% Sox17+ cells and negligible Sox2+ cell population, indicating efficient differentiation from pluripotency.
- 0.2% DMSO exposure reduced Sox17+ population by 5.0%, while endoderm inhibitor, SB43154, perturbed Sox17 expression.
- Assay performance: Z' = 0.77, intra CV = 6.5%, inter CV = 5.8%





Assay Calibration with Reference Chemicals

- Initial testing was done on four reference non-teratogens (top row) and four teratogens (bottom row).
- Teratogens displayed decreased Sox17+ populations, while non-teratogens had no effect; *n* = 8, bars = 2*STD.
- Chemicals that reduced the Sox17+ population below the 0.2% DMSO control median value were deemed a teratogen hit.



Assay Training Set Screen of 66 Chemicals

- Known non-teratogens and teratogens were tested using the assay with a 9-conc. response; *n* = 4. Chemical potency (active concentration at cutoff) and maximum observed inhibition is shown below for each chemical.

- Screening revealed an assay sensitivity, specificity and balanced accuracy of 81%, 63% and 72% respectively for identifying teratogens.
- Future work will include additional screening with directed ectoderm and mesoderm differentiation to investigate if chemical effects impacting differentiation to other lineages can be detected.

References

1) Sei Kameoka, Joshua Babiarz, Kyle Kolaja, Eric Chiao, A High-Throughput Screen for Teratogens Using Human Pluripotent Stem Cells, Toxicological Sciences, Volume 137, Issue 1, January 2014, Pages 76–90, https://doi.org/10.1093/toxsci/kft239
2) Martyn, I., Kanno, T.Y., Ruzo, A. et al. Self-organization of a human organizer by combined Wnt and Nodal signalling. Nature 558, 132–135 (2018). https://doi.org/10.1038/s41586-018-0150-y