Profiling the ToxCast library with pluripotent embryonic stem cell assays

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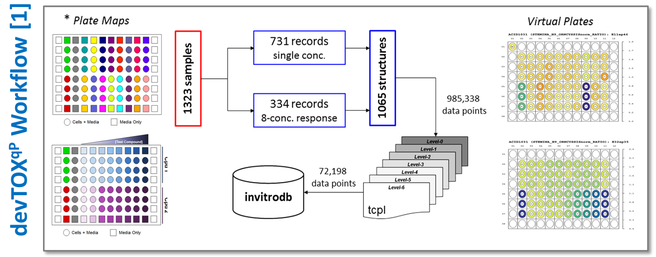
Introduction

ToxCast chemicals were profiled for developmental toxicity potential in two embryonic stem cell assays and processed in the ToxCast data analysis pipeline (tcpl):

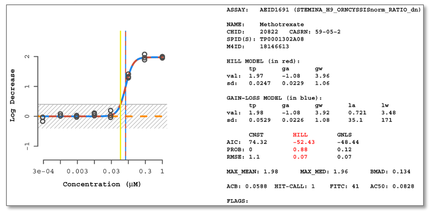
[1] human pluripotent H9 stem cell-based (hESC) assay monitoring a metabolic biomarker [Palmer et al. 2013, BDRB];
[2] mouse differentiating embryonic stem cell (mESC) adherent assay [Barrier et al. 2011, Reprod Tox].

hESC (pluripotent) assay



- ↓ ornithine/cysteine in the day 3 secretome predicts µM threshold for teratogenicity (TI) [1];
- point of departure for cell viability equates to 11% reduction in cell number.

Example: Methotrexate (TI = 0.059 µM)



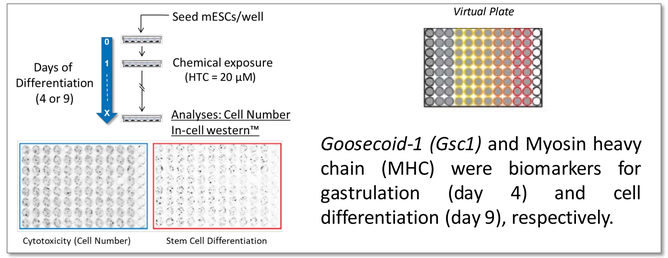
- TI was recorded for 181 chemicals (17% of 1065 tested); model performance used 42 benchmark compounds and ToxRefDB prenatal studies in rats and/or rabbits (dLEL ≤ 200 mg/kg/day) [manuscript in preparation].

hESC model performance

stringency filter applied to the in vivo anchor

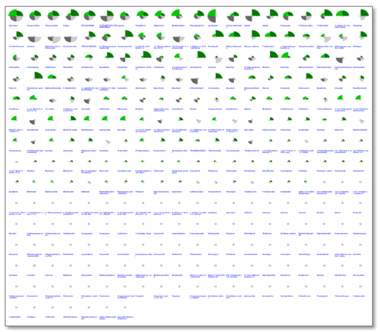
	benchmark	*none*	*low*	*medium*	*high*
TP	17	85	60	35	19
FP	0	14	37	23	9
FN	9	217	127	51	11
TN	16	116	208	176	88
n	**42**	**432**	**432**	**285**	**127**
Sensitivity	0.654	0.281	0.321	0.407	0.633
Specificity	1.000	0.892	0.849	0.884	0.907
Accuracy	78.6%	46.5%	62.0%	74.0%	84.3%
MCC	0.647	0.190	0.202	0.332	0.554

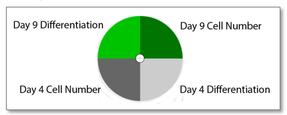
mESC (differentiation) assay



Goosecoid-1 (Gsc1) and Myosin heavy chain (MHC) were biomarkers for gastrulation (day 4) and cell differentiation (day 9), respectively.

Effects of ToxCast chemicals on mESC endpoints





- Combined mESC response on 287 ToxCast chemicals;
- ToxPi slice size indicates effect on each endpoint;
- rank order based on overall potency of response;
- clustering reflects similarity in the response pattern.

mESC model performance

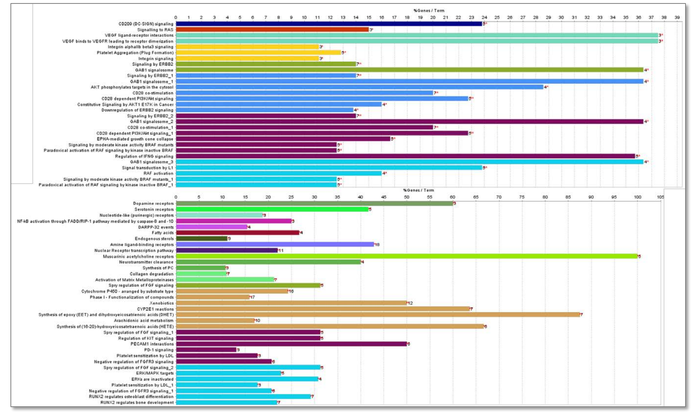
low	*medium*	*high*
95	23	1
40	40	32
126	35	5
54	52	44
315	150	82
0.430	0.397	0.167
0.300	0.565	0.579
47.3%	50.0%	54.9%
0.004	-0.038	-0.135

- positive mESC response recorded for 95 chemicals (30.1% of 315 with ToxRefDB prenatal rat or rabbit studies);
- 221 dLEL-positives: *Gsc1* picked up 28% and MHC picked up 25% (overlap = 11% for *Gsc1* on day 4 + MHC on day 9).

Mining the ToxCast dataset to define assay sensitivity

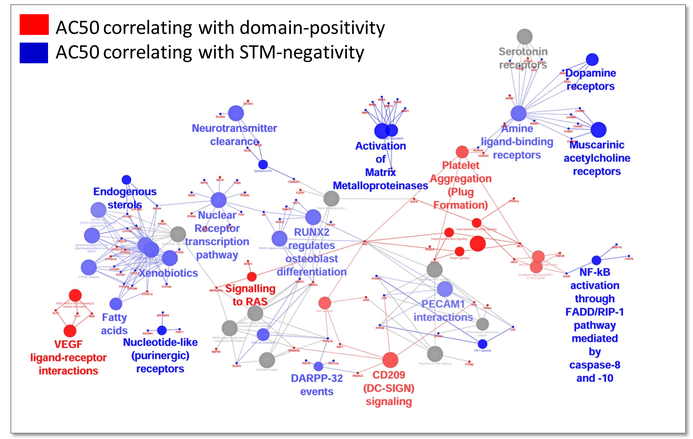
To gain insight into the biological pathways and targets associated with the stem cell responses, machine-learning was used to mine correlations to 337 enzymatic and receptor signaling assays in the ToxCast NovaScreen dataset (NVS). Each NVS assay was enriched for an AC50 correlation against a hESC-positive or hESC-negative outcome, weighted by an assay-specific logistic regression model, processed through the Reactome HSA Pathway Browser (v3.5, database release 63), and independently enriched for significant pathway associations with the ClueGO plug-in to Cytoscape v3.4 (Bonferroni-corrected p ≤ 0.05, minimum 3 genes for a pathway identifier).

Sensitive Domain (86 NVS targets)
Insensitive Domain (131 NVS targets)



Domain Network (hESC):

- enriched pathway interactions mapped with the Cluepedia plug-in to Cytoscape.
- positive-response examples: inhibition of BRAF signaling, adrenocorticoids (GR, MR);
- negative-response examples: female hormone receptors (ESR1, PR), muscarinic receptors (M1-5), MMPs.
- mESC examples: p53 signaling sensitive, IL8 signaling not.



Summary

- ToxCast chemicals were classified for potential developmental toxicity using the hESC devTOX^{qp} platform from Stemina Biomarker Discovery [1] or an adherent mESC assay [2].
- Performance against prenatal animal studies (ToxRefDB) improved from 62% to >84% accuracy as the level of confidence in the *in vivo* anchoring result (dLEL) increased.
- Characterizing the applicability domain at a pathway level sets the stage for new approach methodologies predicting developmental toxicity without vertebrate animal testing.