An integrative systems toxicology model for neurovascular developmental toxicity

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Background

Chemical perturbation of brain development is a children's environmental health concern. Blood-brain barrier (BBB) development is mediated by complex signals commuted in the neurovascular unit (NVU) by diverse cell types of the neuroepithelium (neuroprogenitor cells/radial glia, microglia, pericytes) and invading vasculature (endothelial cells). Here, we describe a computational systems model for the biological regulation of BBB development and demonstrate its implementation toward testing a hypothesis-driven signature of developmental neurovascular toxicity.

Methods

1 Build

- Literature curation identified 86 proteins important for BBB development. Compared percent amino acid sequence similarity across species (SEQApass.epa.gov).
- Mapped protein connectivity to cellular targets with CellDesigner v4.4 and identified nodes (yellow) represented in ToxCast.

2 Classify

- Identified putative BBB toxicants from 1058 ToxCast Phase I/II chemicals based on 23 technical targets corresponding to 98 ToxCast assay features (www.actor.epa.gov).
- Rank-ordered the chemical set based on ToxPi 2.0 (beta, D. Reif, NCSU).

3 Evaluate

- Modeled angiogenic and neurogenic responses to a 38-chemical test set in a collection of human cell-based *in vitro* assays relevant to the BBB.
- Filtered active chemical-associated ToxCast assays as a signature of neurovascular unit (NVU) activity to evaluate *in silico* predictions.

38 ToxCast Chemicals

EXPOSURES: Control: 0.1% DMSO; ArunA and VALA: 0.1 - 100 µM (5 conc.); FICAM: 0.0001 – 500 µM (5+ conc.)

Neurogenesis

Assay source code (A)

hESC (H9) → hNP1, hNC, hNN2 → Cell titer (CT), Cell migration/proliferation (MG), Neurite outgrowth (NC, NL, NN, BN)

Angiogenesis

Assay source code (V)

HUVEC (iPSC (in progress)) → Cell titer (CT); Tubule formation agonist (TUB+); tubule length (TL); Tubule formation antagonist (TUB-); tubule length (TL); Cell migration/proliferation (MG) wound area (wa), β-catenin (bc)

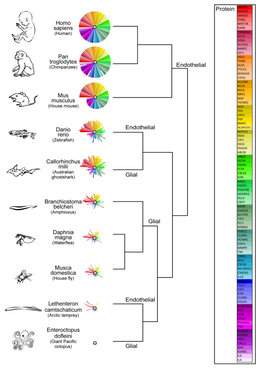
Assay source code (F)

HUVEC → Cell titer (CT); Tubule formation antagonist (TUB-); tubule length (TL)

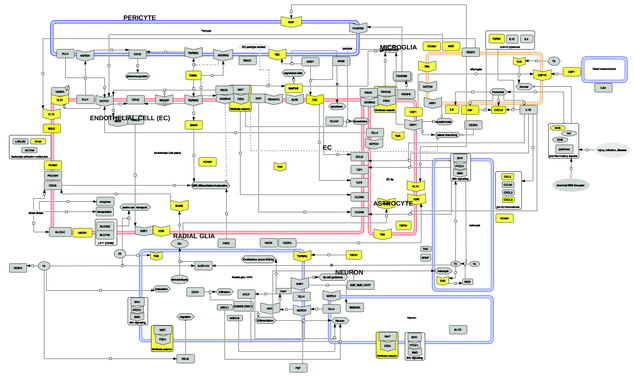
***In vitro* ToxPi analysis:** Chemical clustering was based on transformed (–log10(x)+6) AC50 values (x = µM concentration for each chemical-assay feature) using the Ward.D algorithm in ToxPi 2.0 (beta).
Assay annotation: SOURCE_CELL TYPE_FEATURE_BENCHMARK

Results

1 ~ Building a model of molecular interactions leading to BBB development [1]



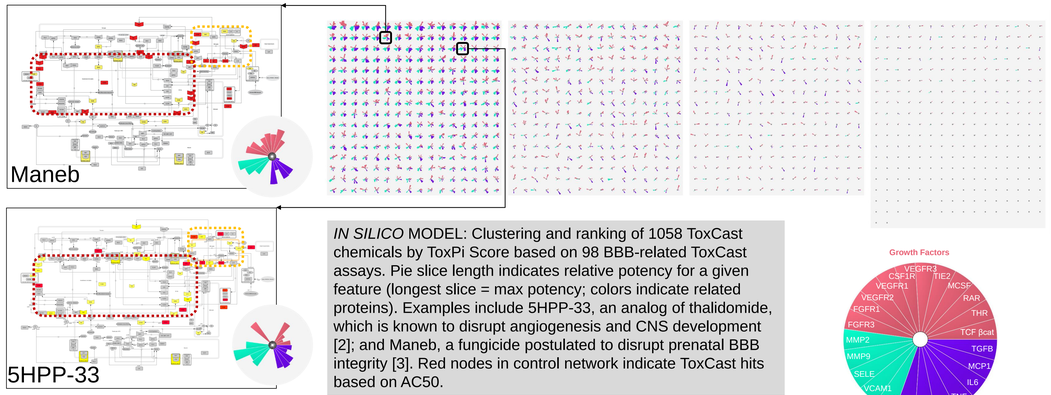
SEQApass and ToxPi v2.0 (beta)



CellDesigner v4.4

BBB DEVELOPEMENT: Phylogeny (left figure) and ontogeny (right figure) of human BBB development.
Phylogeny of the key genes are indicated as a ToxPi from Cephalopods to Primates based on deduced protein sequence (colors for visualization purposes only).The cellular network indicates key interactions by cell type and signal (yellow nodes indicate 23 ToxCast targets with associated assays available).

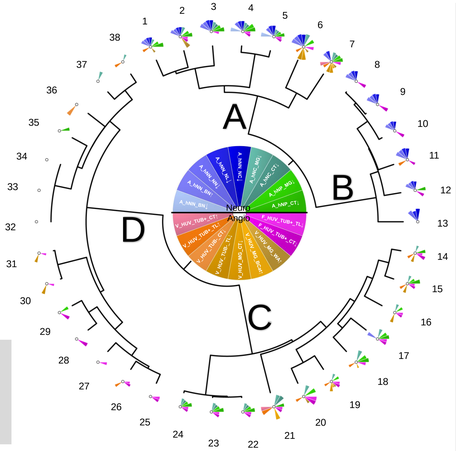
2 ~ Classifying putative developmental neurovascular toxicants by ToxPi score



IN SILICO MODEL: Clustering and ranking of 1058 ToxCast chemicals by ToxPi Score based on 98 BBB-related ToxCast assays. Pie slice length indicates relative potency for a given feature (longest slice = max potency; colors indicate related proteins). Examples include 5HPP-33, an analog of thalidomide, which is known to disrupt angiogenesis and CNS development [2]; and Maneb, a fungicide postulated to disrupt prenatal BBB integrity [3]. Red nodes in control network indicate ToxCast hits based on AC50.

3 ~ Evaluating the neurovascular effects of 38 chemicals represented across ToxPi ranks in classification model

1 Oxytetracycline dihydrate	A
2 Haloperidol	
3 Quercetin	
4 Disulfiram	
5 Celecoxib	
6 Clarithrine	
7 SHPP-33	
8 Methylparaben	B
9 Diethanolamine	
10 Pymetrozine	
11 Methimazole	
12 Valproic acid	
13 PFOS	
14 Reserpine	
15 Docusate sodium	C
16 Phenolphthalein	
17 Triclocarban	
18 Octyl gallate	
19 tert-Butylhydroquinone	
20 Pyridaben	
21 TNP-470	
22 Bisphenol AF	
23 4-Nonylphenol, branched	
24 1-Hydroxypyrene	
25 Triclosan	
26 Sodium dodecylbenzenesulfonate	D
27 Fluazinam	
28 Decane	
29 1,2,3-Trichloropropane	
30 Tris(2-ethylhexyl)phosphate	
31 Bisphenol A	
32 C.I. Solvent Yellow 14	
33 1,2,4-Trichlorobenzene	
34 Imazamox	
35 D-Mannitol	
36 Tris(2-chloroethyl) phosphate	
37 Tris(1,3-dichloro-2-propyl)phosphate	
38 2,4-Diaminotoluene	



Wilcoxon Rank Sum Test Results:
C > D; p = 0.003
A > D; p = 0.015
B > D; p = 0.778

IN VITRO DATA: Chemical clustering based on 9 neurogenesis (blue/green) and 9 angiogenesis (pink/orange) *in vitro* assays. Colors indicate cell type (blue = NN, green = NPC, pink/orange = HUVEC)
Wilcoxon Rank Sum Test: Compared 'NVU-active' chemicals (A, B, and C) to 'NVU-inactive' chemicals (D) based on ToxPi scores from the classification model.

Summary and Conclusions

- A systems state map of BBB development integrated with available ToxCast bioactivity profiles was used to rank-order 1058 chemicals and to identify key events that may account for state-specific susceptibility of BBB development.
- Two groups of 'NVU-active' compounds in the *in vitro* assays were predicted BBB disrupting compounds based on activity in ToxCast assays.
- The classification model did not capture neural network effects.
- Current model limitations include inadequate ToxCast assay coverage, absence of key cells (e.g., microglia and pericytes) from the *in vitro* testing platforms, and a limited *in vitro* chemical test set. Adding these components may qualify the prediction and reduce modeling uncertainty.

References

[1] Saili et al. 2017. Blood-brain barrier development: Systems modeling and predictive toxicology. *Birth Defects Research*
[2] Hallene et al. 2006. Prenatal exposure to thalidomide, altered vasculogenesis, and CNS malformations. *Neuroscience*
[3] Thiruchelvam et al. 2002. Developmental exposure to the pesticides paraquat and maneb and the Parkinson's disease phenotype. *NeuroToxicology*.

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