Computational Toxicology and Prenatal Development

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The views expressed in this presentation are those of the author[s] and do not necessarily reflect the views or policies of the U.S. Environmental Protection Agency.
Embryogenesis: orchestration of cellular complexity

**EMBRYONIC CELL BEHAVIORS**

- cell growth & death
- differentiation & function
- cell motility & adhesion
- clocks & organizers
- genetic signals & responses
- ECM synthesis & remodeling

**CONSEQUENCES OF DISRUPTION**

- incorrect cell number
- missing cell types
- disorganization
- chaos and ataxia
- dysregulation
- loss of mechanical properties
Systems models

- **ToxCast/Tox21**: HTS paradigm enables data-driven chemical prioritization and the capacity for predictive toxicology

- **Need**: computational models that reconstruct the dynamics and mechanics of diverse tissues and complex *in vivo* systems

- **Cell networks**: predicting cell-level behavior is complex enough without the emergent potential of a multicellular system

- **Goal**: develop and use cell agent-based models to help inform prioritization, evaluate mechanisms, and predict toxicity
ToxCast DevTox model

exposure metrics and QSAR

Prenatal Model

dose response and reverse dosimetry

In vivo DEV endpoints (36)

ToxRefDB_rat
ToxRefDB_rabbit

Complex culture / SMOs (179)

human 1° cell co-culture
murine ES cells
zebrafish embryos

Cell-based assays (147)

cellular impedance
cellular properties
trans-activation
NPAs

Biochemical HTS (292)

CYP450s
GPCRs
NRs
Kin-Phs
proteases
TRs / ICs

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Prenatal models

- general idea to mine signatures of DevTox from ToxCast/Tox21 data
  - find significant univariate features (single assay feature to DEV endpoint)
  - build multivariate signatures (multiple features to DEV endpoint)
  - map features to known pathways, processes, phenotypes

- predictive DevTox models completed with ToxCast Phase-I data

  angiogenesis: Kleinstreuer et al. 2011, Env Hlth Persp (in press)
  zebrafish development: Padilla et al. (submitted)
  zebrafish concordance: Sipes et al. 2011, Birth Defects Res C (in press)
ToxCastDB: AC50s for 309 Phase-I chemicals for 662 *in vitro* features

ToxRefDB: 17 endpoints in pregnant rats (251) and rabbits (234)

- assays aggregated and reduced by genes/pathways/processes
- linear model assessment with five-fold cross-validation on 80/20 split

**SOURCE:** Sipes et al. 2011 *Toxicol Sci* (in press)
Global prenatal model

Model predicts DevTox in rats with >70% BA

Model predicts predict DevTox in rabbits with >70% BA

Positive predictors
Negative predictors

Prioritization
Features mapped by GO-process

univariate DevTox features
multivariate DevTox features
HTS detected pathway

**ToxRefDB_prenatal**

- **rat**
  - GPCR.pos
  - TGFb.pos
  - RAR.pos
  - MT.pos
  - SENS_CYP.pos
  - AP1.pos
  - SLCO1B1.pos
  - CYP.pos
  - HLA-DR.neg

- **rabbit**
  - CCL2.pos
  - IL.pos
  - PGE2.neg
  - SULT2A1.neg
  - CYP.pos
  - TGFb.pos

**mESC growth & differentiation**

- **Hitra_assay(1)**
- **AP1_assay(1)**
- **ABCG2_assay(2)**
- **ABCB1_assay(1)**
- **Nrf2_assay(1)**
- **Mitochondrial Stress**
- **CRE_assay(1)**
- **p53_assay(2)**
- **H2AX_assay(2)**

**angiogenesis**

- **CXCL10_up**
- **CCL2_down**
- **uPAR**
- **PAI1**
- **Tie2**
- **VEGFRII_down**
Angiogenesis & Vascular disruption
Cell-agent-based models

- stochastic cellular behaviors
- specified cellular activities
- PDE solvers for biochemical gradients
- toolbox of morphogenetic processes
- executes collective cell behavior
- enables emergent properties
Simple CC3D model

macrophage navigating RBCs toward a microbial pathogen

simple CompuCell3D model

SOURCE: M Rountree, NCCT
Angiogenesis is more complex

<table>
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<th>Molecular Signal</th>
<th>Expression</th>
<th>Chemotaxis</th>
<th>Proliferation</th>
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<td>KDR (VEGFR2)</td>
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- **Endothelial Stalk (ECs)**
- **Endothelial Tip (ECT)**
- **Mural Cell (MC)**
- **Inflammatory Cell (IC)**
Angiogenetic field at time=0

SOURCE: Kleinstreuer et al. (in preparation)
Virtual angiogenesis

VEGF 165

Ang1/Tie2

MMPs

CXCL10

VEGF 121

CCL2

sFlt1
Vascular tree, 2-day quail embryo (A,B) [Herrero and Kohn (2009) M3AS]

CC3D Simulation Results (C,D)

- Endothelial Stalk
- Endothelial Tip
- Mural Cell
- Inflammatory Cell

In situ

In silico

VEGF gradient

Normal phenotype
Emergence

Time ➔

- Endothelial Stalk
- Endothelial Tip
- Mural Cell
- Inflammatory Cell

Fentin et al. (2010) Blood

In vitro

Emergence (bridging)

In silico

[Confocal z-stack images showing changes from 11.5 dpc to 12.5 dpc]
TEST CASE: Thalidomide and 5HPP-33

>2-fold change

5 µM

40 µM

Up

Down

CCL2
CCL2 (thal)
upAR
upAR (thal)
Mural Proliferation
IC Proliferation
VCAM1
CXCL10
PAI-1
PAI-1 (thal)
upAR
VEGFR2
**TEST CASE:** Thalidomide and 5HPP-33

**In vitro**


**In silico**

*SOURCE:* Kleinstreuer et al. 2011, in preparation (ToxCastDB, 40 uM)

quantitative prediction emergent from ToxCast HTS data input (AC50s)
Integration (in progress)

**Chick limb** +**CPS49**

**Virtual limb**  **PCD in AER**

Thalidomide induces limb defects by preventing angiogenic outgrowth during early limb formation

Christina Therapontos², Lynda Erskine, Erin R. Gardner, William D. Figg, and Neil Vargesson

Therapontos et al. PNAS 106: 8573-8578, 2009

Rountree et al., 2011 (in preparation)
endothelial connectivity (plexus), in-degree (branching), vessel uniformity (width), sprouting

weakened mural adhesion to nascent vessels; altered endothelial growth and spreading behavior, lack of sprouting due to Flt1 inhibition

endothelial hyperplasia with decreased cell migration and polarization possibly due to increased uPAR – enhances ECM locking

little to no vessel formation
Virtual mixture

Total Cell Number

- Control: 870.1428571
- Mixture: 620.7142857
- Average (multiple simulations): 697.5714286, 677.1428571
Goal: applying HTS data, *in silico* tools, and models to look globally at developmental processes and toxicities in a new way

Approach: predictive and mechanistic models that dynamically integrate data with relevant information about embryonic systems

Virtuomics: run ‘what-if’ scenarios to predict adverse outcomes from different perturbations (chemicals, concentrations, mixtures)

Benefit: scientifically-based predictions on how development might be affected across a range of complex factors

http://www.epa.gov/ncct/v-Embryo/
Acknowledgements

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