Computational Modeling of Limb-Bud Dysmorphogenesis: Predicting Cellular Dynamics and Key Events in Developmental Toxicity with a Multicellular Systems Model

BK Ahir[1], ES Hunter[2], NC Baker[3], RM Spencer[3], RS Dewoskin[4], TB Knudsen[1]

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

[1]National Center for Computational Toxicology, [2]National Health and Environmental Effects Research Laboratory, [3]Lockheed Martin, [4]National Center for Environmental Assessment

Tom Knudsen | knudsen.thomas@epa.gov | 919-541-9776

1. COMPUTATIONAL EMBRYOLOGY & PREDICTIVE TOXICOLOGY

HYPOTHESIS:

a computer model that simulates cellular function in a growing embryo can be used to predict the potential impact of chemical exposure during early limb development.

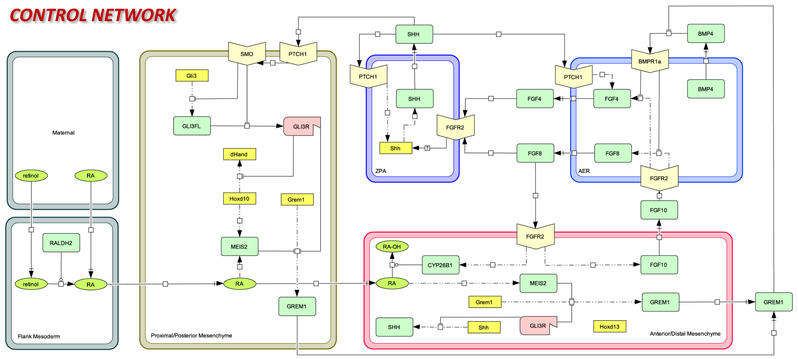
2. SIGNALING NETWORK: *spatial information processing*

Query of Mouse Genome Informatics database (www.informatics.jax.org/) by 'abnormal limb bud morphology' (MP:0005650) returned genes for 132 relevant genotypes.

An *e*-Library developed from these genes (31,701 PubMed records annotated by MeSH terms for limb development) returned 81 genes regulating early limb morphogenesis.

Apical Ectodermal Ridge (AER) and Zone of Polarizing Activity (ZPA) are major signaling centers; pivotal signaling pathways fibroblast growth factor (FGF), sonic hedgehog (SHH), bone morphogenetic protein (BMP) and retinoic acid (RA) were modeled in CellDesigner (www.celldesigner.org/).

CONTROL NETWORK

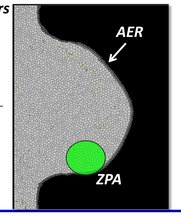


3. CELLULAR DYNAMICS: *translation of spatial information*

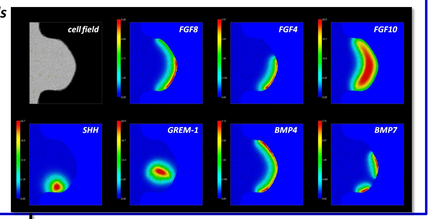
CELL AGENT-BASED MODEL (ABM): multicellular and signaling dynamics were modeled in *CompuCell3D* (www.compucell3d.org/); the small working prototype simulated mouse hindlimb-bud development between Theiler stages 16-19 (~42h) in ~42,000 Monte Carlo Steps (MCS).

Cellular behaviors

- *Adhesion*
- *Apoptosis*
- *Differentiation*
- *Migration*
- *Mitosis*
- *Shape*
- *Size*

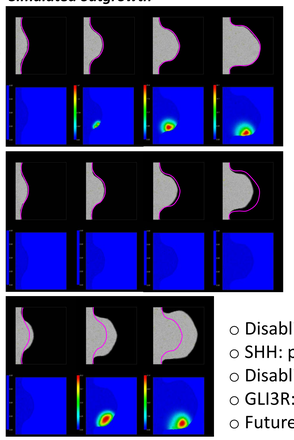


Signals

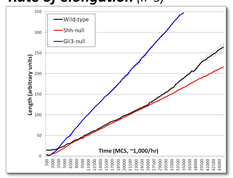


GENETIC PERTURBATION: hindlimb-bud shown at MCS intervals 10k, 20k, 30k & 40k with SHH field for normal and 'virtual knockouts' disabled for *Shh* or *Gli3* genes (*wild-type contour traced magenta).*

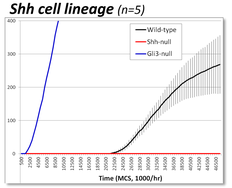
Simulated outgrowth



Rate of elongation *(n=5)*



Shh cell lineage *(n=5)*



Predicted outcomes

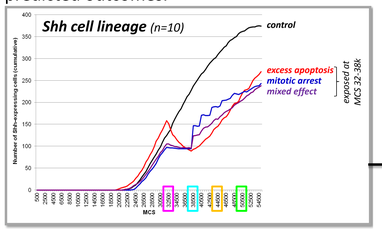
digital patterns inferred from the literature; not yet implemented in the model

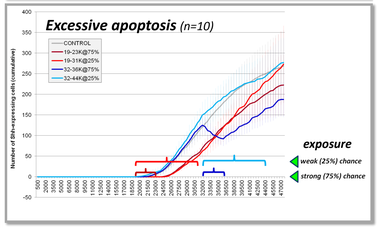
- Disabling *Shh* in the model simulates undergrowth of limb-bud
- SHH: posterior regulation of digit number & identity
- Disabling *Gli3* in the model leads to precocious overgrowth
- GLI3R: blocked by SHH but negatively regulates *Shh*-expression
- Future Goal: Model the emergence of digital rays

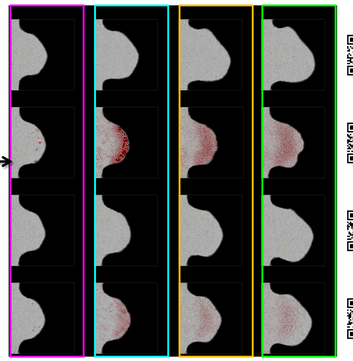
4. TOXICODYNAMICS: *predicting key events*

CHEMICAL DISRUPTION: How might local effects predicted by *in vitro* high-throughput screening (HTS) data such as ToxCast™ propagate through the pivotal SHH cell lineage *in silico* to predict, therefore, a key event *in vivo*?

EXAMPLE: 5-Fluorouracil, a teratogen that disrupts digit formation, perturbed 13 of 650 ToxCast HTS assays at ≤ 15 µM: impaired differentiation and increased cell loss (excessive apoptosis); p53-induction, mitotic arrest and cell death. These effects can be fed into the model for translation into predicted outcomes.







scan QR codes to run simulation on mobile device

v-Embryo™

- Model reacts quantitatively to transitive mitotic arrest and/or excessive apoptosis
- Virtual models can rapidly sweep many 'what-if' exposure scenarios to predict response

5. PRACTICAL APPLICATIONS OF A MULTICELLULAR SIMULATION

- **Predictive Toxicology :-** new way to integrate masses of HTS data into knowledge of a system
- **Parameter Sweeps :-** high-throughput hypothesis testing to inform experimental design
- **Mechanistic Models :-** track lesion propagation through higher levels of biological organization
- **Temporal Analysis :-** pinpoint key events and mine for quantitative relationships
- **Dose Predictivity :-** compare diverse exposure scenarios across dose, time, duration & mixtures