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

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Congenital limb malformations are among the most frequent malformation occurs in humans, with a frequency of about 1 in 500 to 1 in 1000 human live births. ToxCast is profiling the bioactivity of thousands of chemicals based on high-throughput (HTS) and computational methods that integrate knowledge of biological systems and in vivo toxicities (www.epa.gov/ncct/toxcast/). Many ToxCast assays assess signaling pathways and cellular processes critical to development, creating the opportunity for computational models that predict these effects in the embryo. Computer simulation of cellular networks is one possible solution for modeling key events in developmental toxicology. We constructed a multicellular agent-based model (ABM) of early limb-bud development in the CompuCell3D (www.compucell3d.org/). The model simulates key cellular behaviors (mitosis, apoptosis, adhesion, migration, chemotaxis, shape, secretion), organizing centers (AER, ZPA) and their signals (FGFs, SHH, BMPs, RA). It effectively emulates hindlimb-bud development during a 42h period in mouse (Theiler stages 16-19) and 160h in human (Carnegie stages 13-16). The ABM reflects biological variability across parallel simulations for spatio-temporal expression of biochemical gradients and cell behaviors (eg, mitosis, apoptosis), ultimately manifesting in measureable trajectories of outgrowth. To evaluate the model as a tool for predictive toxicology, we selected trans retinoic acid as a prototype limb teratogen and 5-Fluorouracil (5-FU) (as a reference compound). 5-FU perturbed 13 of 650 ToxCast assays based on AC50s (or LECs) at or below 15 uM. 5-FU effects observed in the assays were disruption of stem cell (mES) growth and differentiation, suppression of TGF-β1 signaling and mitochondrial density, p53-induction, mitotic arrest, reduced cell proliferation and increased cell death. Challenging the ABM with concentration-response data derived from mES cell number produced a dose-dependent wave of mitotic arrest and apoptosis, disrupting outgrowth. Varying the dose and time of exposure localized the primary key event to arrest of SHH-expressing cells and their geometric relationships to cells expressing GREM1, a BMP antagonist maintained by SHH signals. Different outcomes emerged when perturbation of the SHH/GREM1/BMP loop was switched between mitotic arrest and excessive apoptosis, indicating the importance of considering both cellular consequences together. These findings support the application of multi-cellular ABMs as tools to translate cellular dynamics into simulation of emergent (higher order) tissue effects for predictive toxicology. [This abstract does not necessarily reflect EPA policy.]