United States Environmental Protection Agency

Computational Toxicology and Short-term Assays for Identifying Developmental Toxicants

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Disclaimer: views are those of the presenter and do not necessarily reflect Agency policy nor imply endorsement of software used here

Office of Research and Development
National Center for Computational Toxicology

Human Health Hazards Indicators, Sacramento
March 15-16, 2010

United States Environmental Protection Agency

http://www.epa.gov/ncct/toxcast/

EPA
NTP
NCGC

Tox21

- project to profile the biological activity of thousands of environmental chemicals using *in vitro* HTS assays,
- identify mechanisms in high-throughput and prioritize chemicals for more extensive toxicological evaluation
- build predictive computational (*in silico*) models of *in vivo* biological response (e.g., developmental toxicity)

Developmental Toxicity

WHAT: chemical exposure during formative stages of life affecting children's health

WHY: we need to predict and understand how chemical compounds disrupt:

- prenatal development (birth defects)
- postnatal function (developmental disabilities)
- pubertal development (impaired fertility)

HOW: biological profiling, bioinformatics, systems biology and computational models

ToxCast™ *in vitro* profiling

Biochemical HTS assays

- Protein families
  - GPCR
  - NR
  - Kinase
  - Phosphatase
  - Protease
  - Other enzyme
  - Ion channel
  - Transporter
- Assay formats
  - Radioligand binding
  - Enzyme activity
  - Co-activator recruitment

309 chemicals
471 assays

Cell-based assays

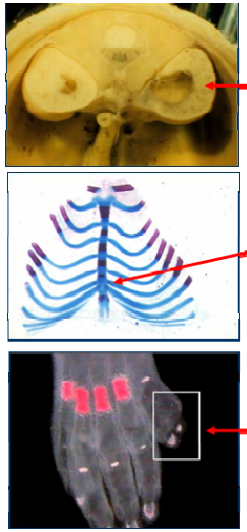
- Cell lines
  - HepG2 human hepatoblastoma
  - A549 human lung carcinoma
  - HEK 293 human embryonic kidney
  - **J1 mouse ES cells (ACDC)**
- Primary cells
  - Human endothelial cells
  - Human monocytes
  - Human keratinocytes
  - Human fibroblasts
  - Human proximal tubule kidney cells
  - Human small airway epithelial cells
- Biotransformation competent cells
  - Primary rat hepatocytes
  - Primary human hepatocytes
- Assay formats
  - Cytotoxicity
  - Reporter gene
  - Gene expression
  - Biomarker production
  - High-content imaging for cellular phenotype

Profiling developmental toxicity

http://www.epa.gov/NCCT/toxrefdb/

ToxRefDB includes:

751 prenatal studies (mostly rat, rabbit)
387 chemicals (283 for both species)
293 DEV endpoints (18 target systems)
194 ToxCast 'positives'

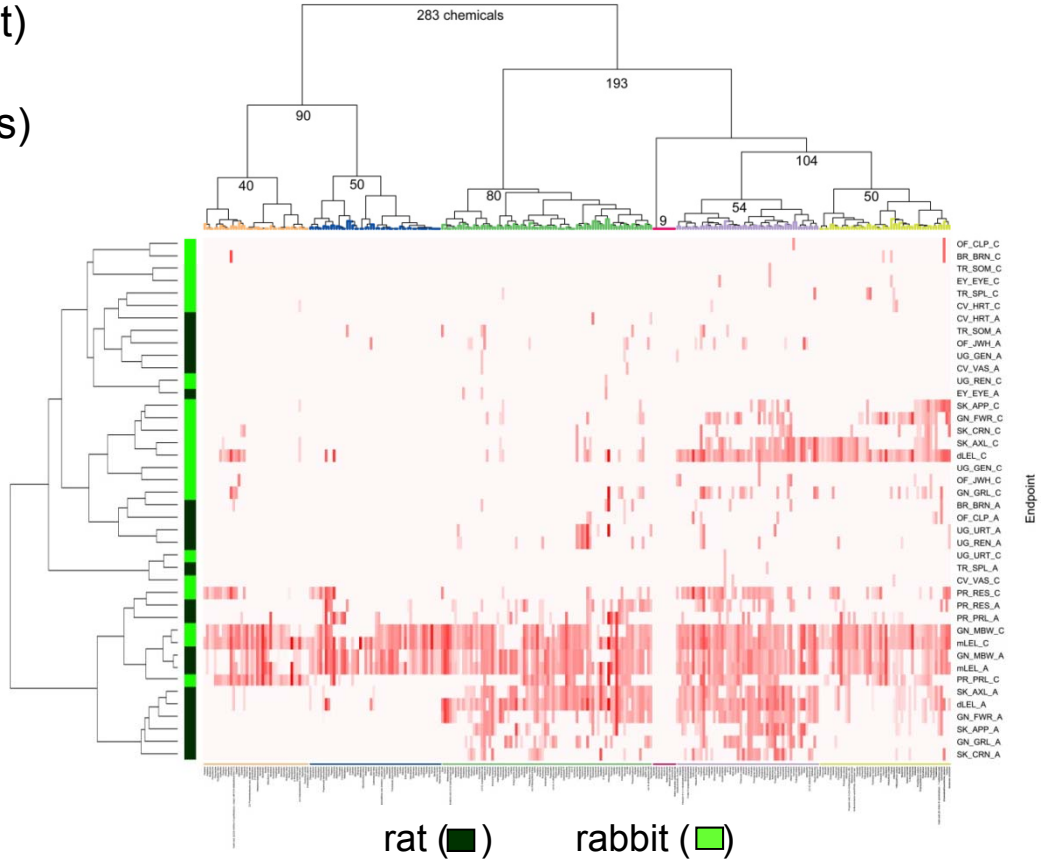


images from www.DevTox.org

target: kidney
description: absent renal papilla
code: UG_REN_3.1060.5013

target: sternebra
description: incomplete ossification
code: SK_AXL_2.1099.5130

target: hindpaw
description: polydactyly (digit I)
code: SK_APP_2.1051.5234



SOURCE: Knudsen et al. (2009) Repro Toxicol 28: 209-219

Prenatal effects in ToxRefDB:

194 ToxCast™ chemicals

CRITICAL ENDPOINT	NUMBER of CHEMICALS		
	rabbit	***rat***	***overlap***
Developmental (global)	111	153	70
Skeletal_Axial	55	118	18
FetalWeightReduction	49	92	6
Skeletal_Appendicular	24	50	7
Skeletal_Cranial	21	41	1
Embryo-Fetal losses	33	35	5
Urogenital (renal, ureteric)	3	19	0
JawHyoid	8	14	0
CleftLipPalate	2	11	0
Neurosensory (brain and eye)	6	8	0
BodyWall (somatic)	1	6	0
Viscera (splanchnic)	9	4	0
Cardiovascular (heart, major vessels)	6	3	0

SOURCE: http://www.epa.gov/NCCT/toxrefdb/

Workflow: ToxMiner v16

ToxCast™ HTS data (AC50s)

	(+)ve	(-)ve
(+)ve	TP	FP
(-)ve	FN	TN

ToxRefDB™ DEV data (cLELs)

UNIVARIATE ASSOCIATIONS

- *t-test (continuous assay data)*
- *classifier (2x2 contingency table)*
- *Fisher's exact test (dichotomous data)*

MULTIVARIATE MODELS

- *machine learning, 80/20 split*
- *5- or 10-fold cross-validation*
- *sensitivity analysis (ROC curves)*

MULTICELLULAR MODELS

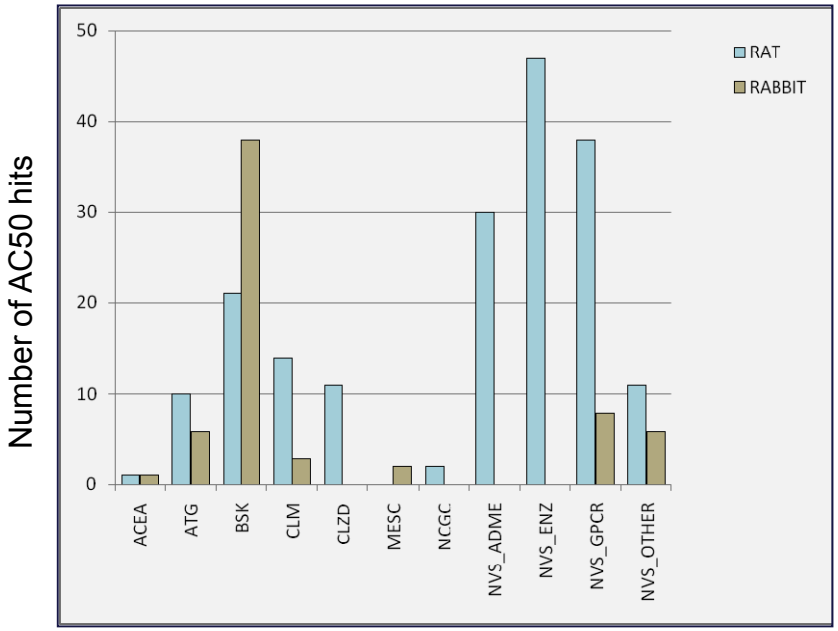
- *knowledge-base (VT-KB)*
- *computer simulations (VT-SE)*
- *biological inferences / mechanisms*

1. Univariate associations

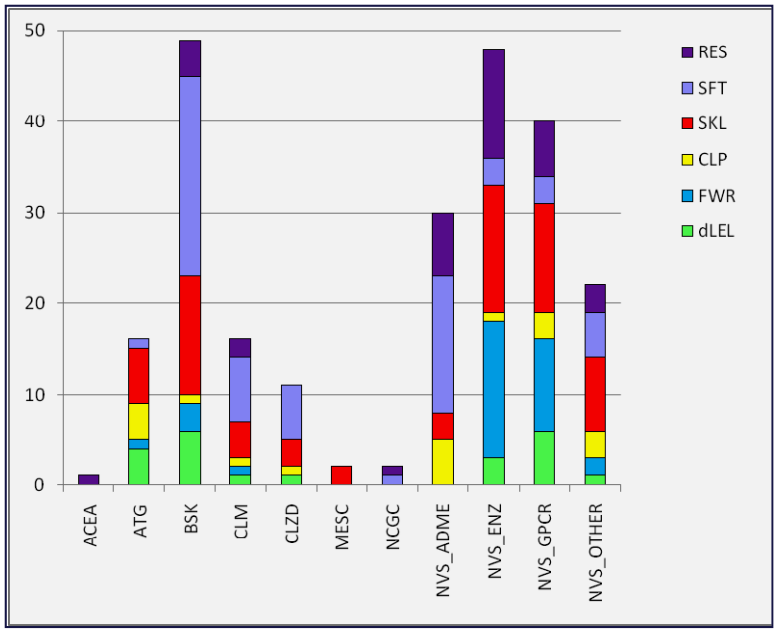
distribution by assay platform

~1% of univariate associations in ToxMiner v16 were DEV-linked

Aggregated by species



Stratified by system

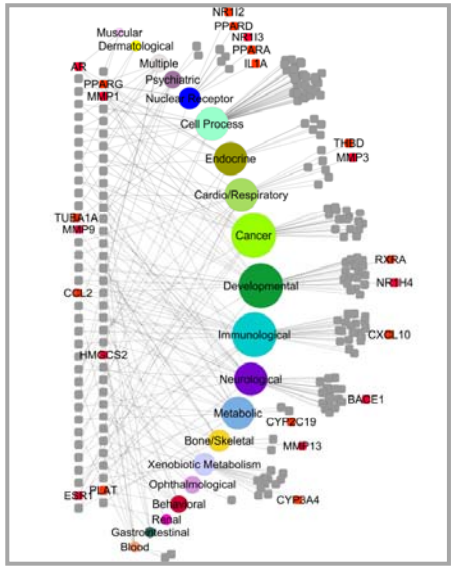


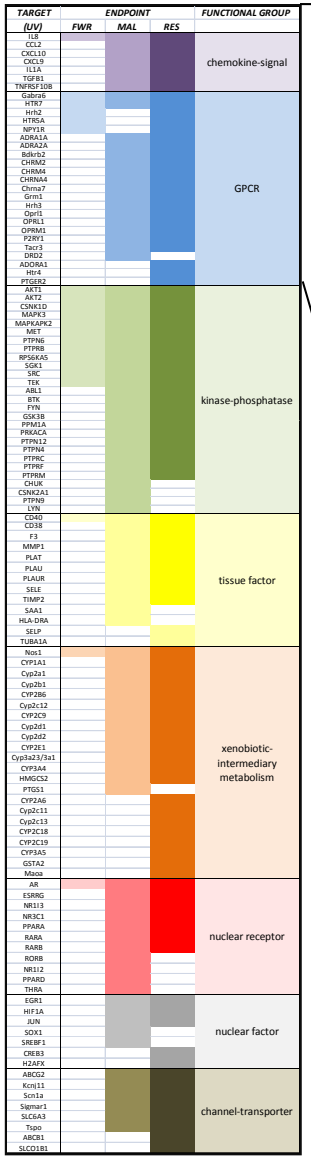
SOURCE: http://www.epa.gov/NCCT/toxcast/

AC50 targets: gene symbol

target ***assays*** (154 nonredundant) mapped to 'penetrance spectrum'

target ***pathways*** inferred from perturbation score



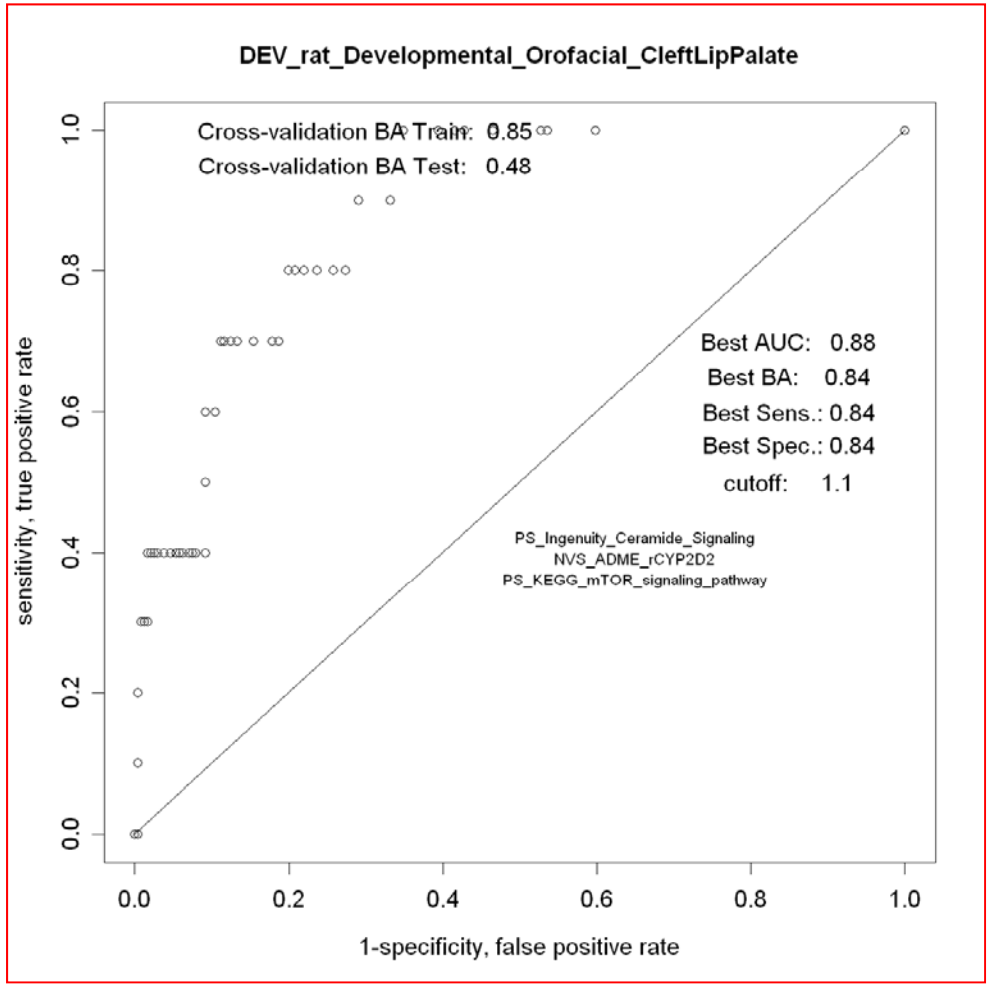


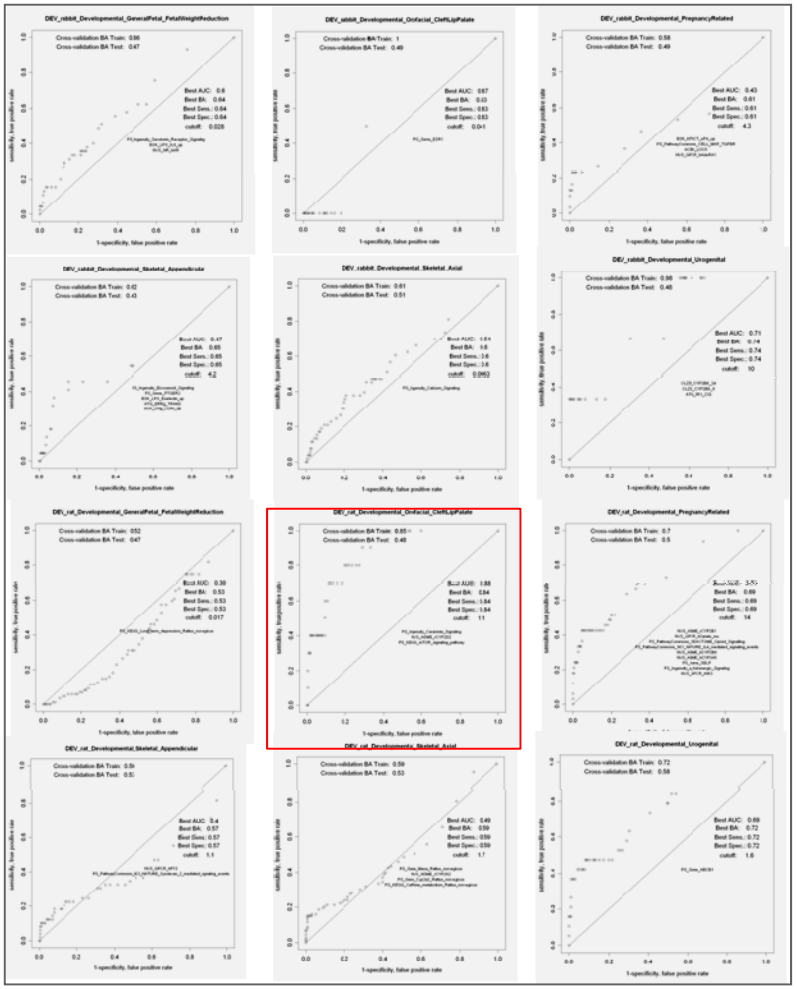
TARGET (UV)	ENDPOINT FWR	ENDPOINT MAL	ENDPOINT RES	FUNCTIONAL GROUP
IL8				chemokine-signal
CCL2				
CXCL10				
CXCL9				
IL1A				
TGFB1				
TNFRSF10B				
Gabra6				GPCR
HTR7				
Hrh2				
HTR5A				
NPY1R				
ADRA1A				
ADRA2A				
Bdkrb2				
CHRM2				
CHRM4				
CHRNA4				
Chrna7				
Grm1				
Hrh3				
Oprl1				
OPRL1				
OPRM1				
P2RY1				
Tacr3				
DRD2				
ADORA1				
Htr4				
PTGER2				

	FWR	MAL	RES
assays	21	98	104
pathways	75	70	11

SOURCE: H Mortensen, R Judson NCCT 2009

2. Multivariate models





SOURCE: N Kleinstreuer , NCCT 2010

Example: inputs to ‘cleft palate’ learner

Assay_1	*Assay_2*	*TP*	*FP*	*FN*	*TN*	*p(max)*
Apoptosis	rat_CLP	7	82	4	167	0.095366
Cell_Cycle_G1S_Checkpoint_Regulation	rat_CLP	8	97	3	152	0.116864
CELL_MAP_AndrogenReceptor	rat_CLP	10	137	1	112	0.065042
CELL_MAP_TGFBR	rat_CLP	11	165	0	84	0.030038
Ceramide_Signaling	rat_CLP	10	118	1	131	0.148738
ERKMAPK_Signaling	rat_CLP	11	152	0	97	0.101302
Estrogen_Receptor_Signaling	rabbit_CLP	2	86	0	156	0.129124
FOXA_transcription_factor_networks	rat_CLP	10	151	1	98	0.055945
FOXM1_transcription_factor_network	rabbit_CLP	2	82	0	160	0.117588
Innate_Immunity_Signaling	rat_CLP	8	89	3	160	0.110672
KEGG_Cell_cycle	rat_CLP	8	93	3	156	0.111922
KEGG_mTOR_signaling_pathway	rat_CLP	5	21	6	228	0.112492
KEGG_Neuroactive_ligand_receptor_interaction_Rattus_norvegicus	rat_CLP	8	76	3	173	0.032864
KEGG_Renal_cell_carcinoma	rat_CLP	10	132	1	117	0.02988
KEGG_Wnt_signaling_pathway	rat_CLP	9	104	2	145	0.116546
PDGFR_alpha_signaling_pathway	rat_CLP	9	94	2	155	0.060048
PPARaRXRa_Activation	rat_CLP	11	184	0	65	0.070501
Presenilin_action_in_Notch_and_Wnt_signaling	rat_CLP	9	95	2	154	0.085948
RAR_Activation	rat_CLP	10	147	1	102	0.054288
S1P2_pathway	rat_CLP	8	89	3	160	0.127029
Signaling_by_TGF_beta	rat_CLP	7	68	4	181	0.065642
Signaling_events_mediated_by_HDAC_Class_I	rat_CLP	11	138	0	111	0.045469
Signaling_events_mediated_by_HDAC_Class_II	rabbit_CLP	2	86	0	156	0.129124
Signaling_events_mediated_by_PRL	rat_CLP	5	30	6	219	0.063597
Signaling_events_regulated_by_Ret_tyrosine_kinase	rat_CLP	10	119	1	130	0.149391
Signaling_mediated_by_p38_alpha_and_p38_beta	rat_CLP	10	138	1	111	0.066863
Sphingosine_1_phosphate_S1P_pathway	rat_CLP	10	122	1	127	0.13399
Toll_Like_Receptor_3_TLR3_Cascade	rat_CLP	8	89	3	160	0.110672
Toll_like_Receptor_Signaling	rat_CLP	8	94	3	155	0.148722
Toll_Receptor_Cascades	rat_CLP	8	89	3	160	0.110672
TRAF6_Mediated_Induction_of_the_antiviral_cytokine_IFN_alphabet	rat_CLP	8	89	3	160	0.110672
Trk_receptor_signaling_mediated_by_the_MAPK_pathway	rat_CLP	5	30	6	219	0.084657
VEGF_Signaling	rat_CLP	5	27	6	222	0.129374
VEGFR1_specific_signals	rat_CLP	5	23	6	226	0.091649
Wntbeta_catenin_Signaling	rat_CLP	10	121	1	128	0.050264

SOURCE: Judson et al, MS in preparation

Hypothesis:

Multiple components in vascular development are potential targets for ToxCast™ chemicals

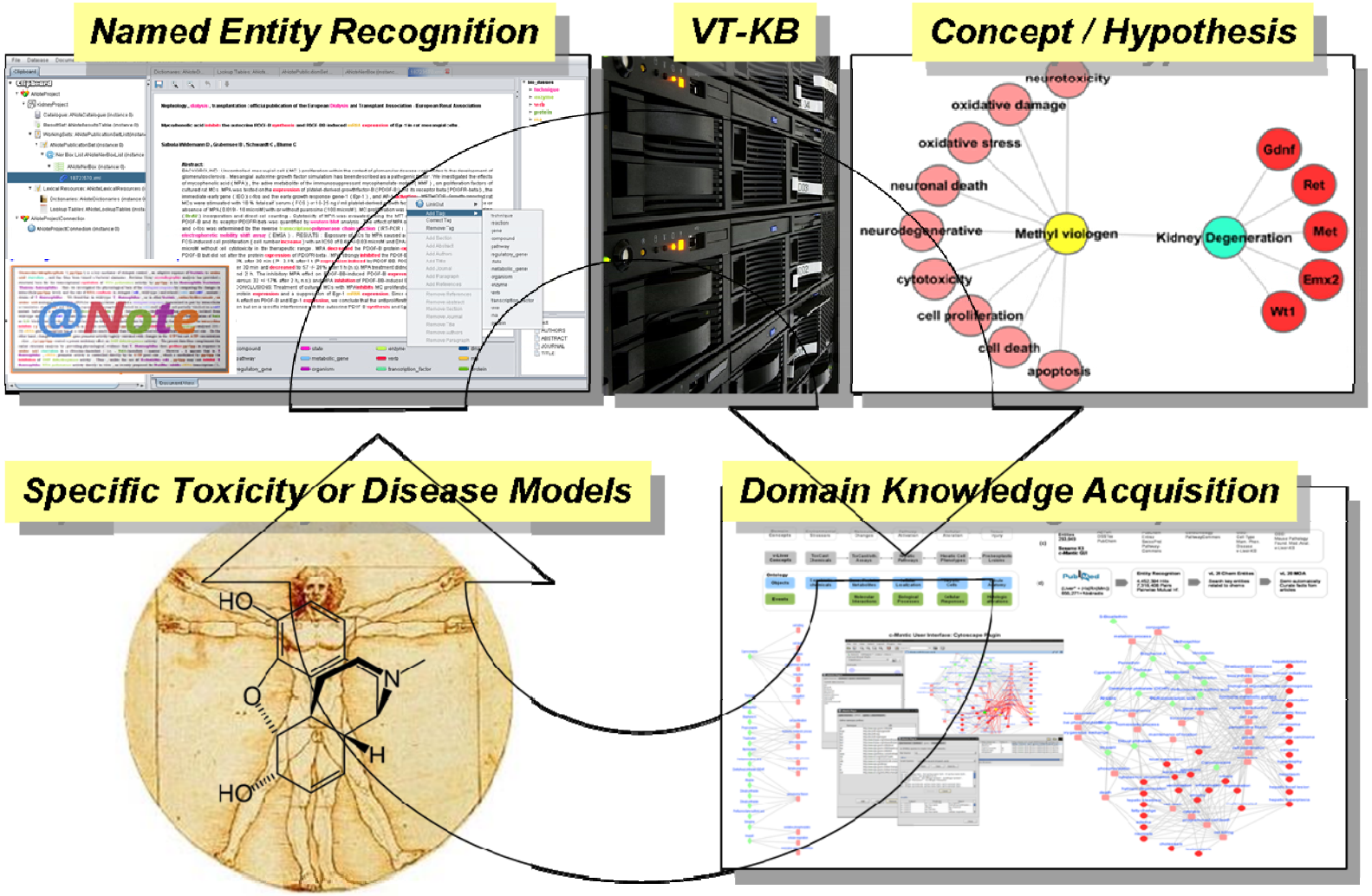
corollary 1:

in vitro bioactivity profiles can be used to mine a 'toxicity signature' for *in vivo* vascular development

corollary 2:

in vitro signature can inform mechanistic models during chemical disruption of embryonic vascular development

Literature mining



SOURCE: Imran Shah, NCCT 2009
Amar Singh, Lockheed Martin

Key formative processes:

vasculogenesis / angiogenesis

- endothelial proliferation, cell migration & chemotaxis
  - RTK signaling: VEGF, VEGF-r, TIE2
  - chemokine signaling: CCL2, CXCL10
- neovascular stabilization:
  - endothelial cell (EC) adhesion
  - vascular smooth muscle cell (VSMC) recruitment
- extracellular matrix (ECM) degradation
  - plasminogen activating system (PAS)
  - matrix metalloproteinases (MMPs)

ToxCast™ inclusion criteria:

weighted assay score

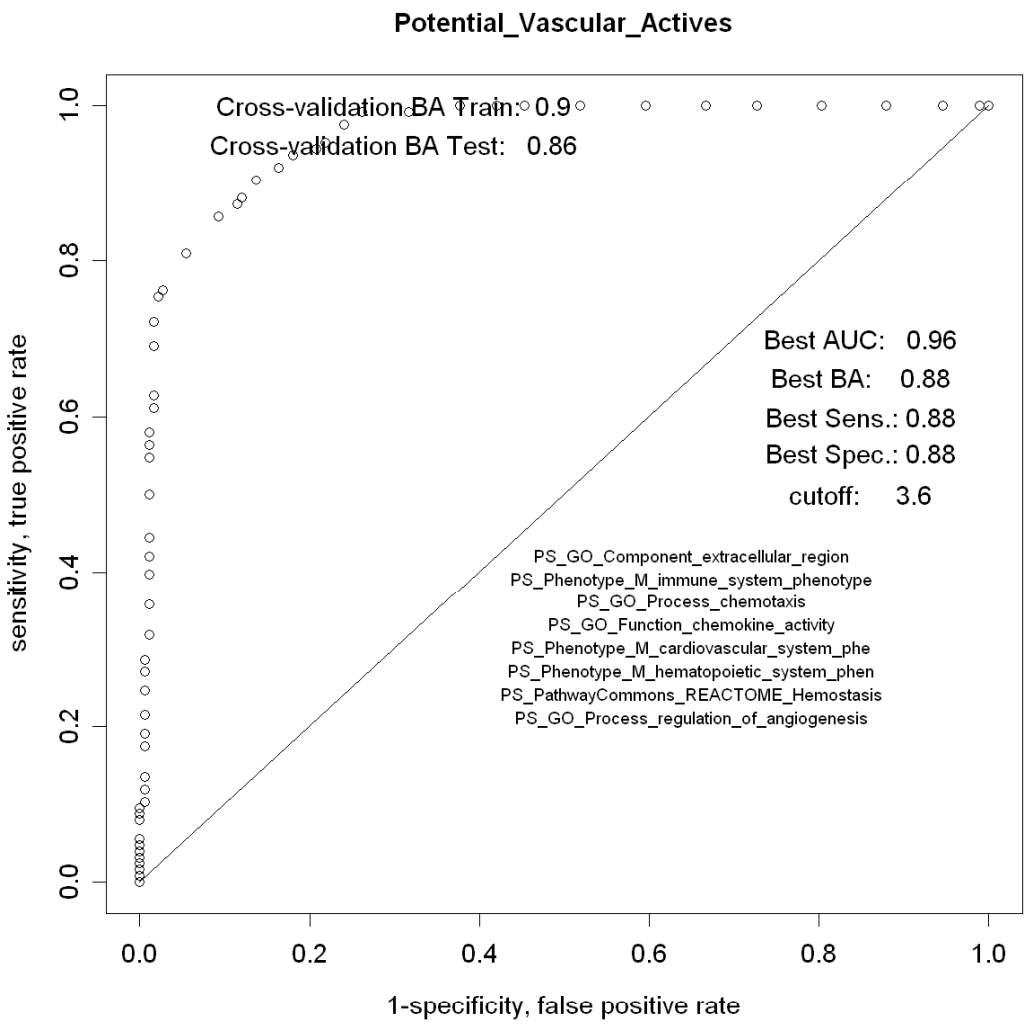
Targets selected by literature co-occurrence with 'vasculogenesis' & 'angiogenesis':

- **decrease** **VEGFR2** receptor (KDR/FLK1)
- **decrease** biochemical **Tie2** activity
- **increase** of **CXCL10** (pro-inflammatory, anti-angiogenic)
- **decrease** of **CCL2** (pro-angiogenic chemokine)
- **increase** **or** **decrease** of PAS via **uPAR** or **PAI1**/SERPINE1

chemicals ranked by weighted signature score, $\Sigma\ k_i{*}AS_i$
where AS = [-log10(AC50(μM)/100000)]

SOURCE: N Kleinstreuer, NCCT 2010

‘Vascular Disruptor’ signature



VDCs

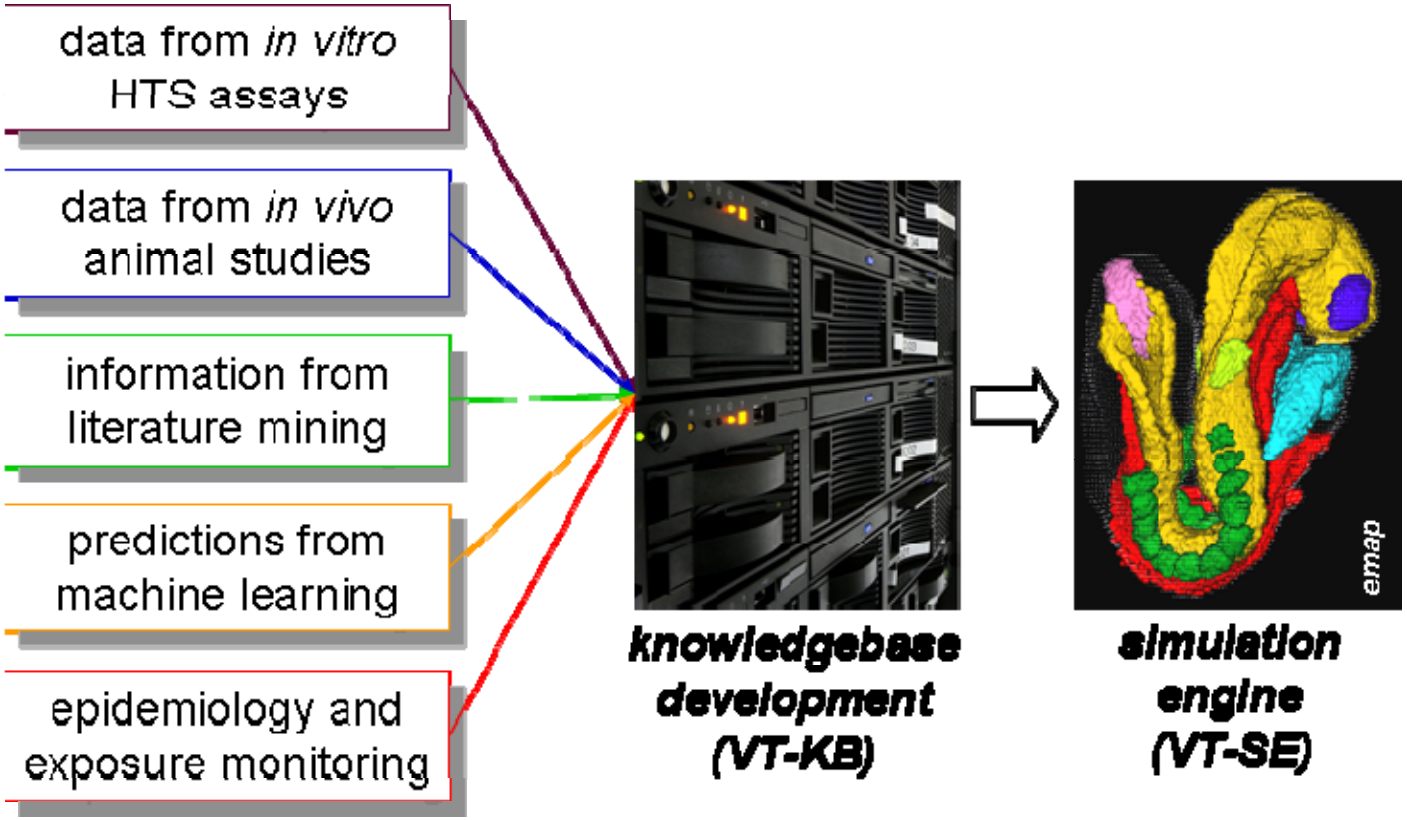
ToxRefDB developmental defects for putative '***Vascular Disruptor Chemicals***'

- *~81% putative VDCs with in vivo data showed developmental effects (eg, FWR, SKL, RES)*
- *~7.5% showed no prenatal effects in rat & rabbit*

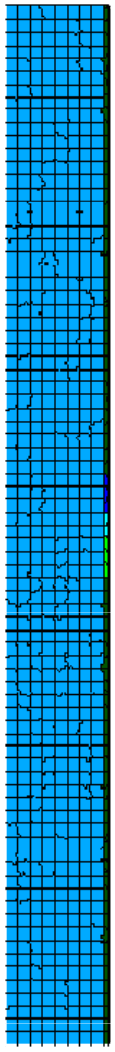
Thiram	;DEV_Rat_General_FetalWeightRedu	No Developmental Rabbit Stud
(Z,E)-Fenpyroximate	;DEV_Rat_Skeletal_Axial	No recorded effect
Emamectin benzoate	;DEV_Rat_General_FetalWeightRedu	No recorded effect
Maneb	;DEV_Rat_General_FetalWeightRedu	No Developmental Rabbit Stud
Fluoxastrobin	;DEV_Rat_Skeletal_Appendicular	No recorded effect
Diniconazole	;DEV_Rat_General_GeneralFetalPath	No recorded effect
Tebufenpyrad	;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelatec
S-Bioallethrin	;DEV_Rat_PregnancyRelated_Embryc	;DEV_Rabbit_PregnancyRelatec
Myclobutanil	;DEV_Rat_Skeletal_Axial	;DEV_Rabbit_PregnancyRelatec
Cyazofamid	;DEV_Rat_Skeletal_Axial	No recorded effect
Benomyl	;DEV_Rat_General_FetalWeightRedu	;DEV_Rabbit_Urogenital_Renal
Tetraconazole	;DEV_Rat_General_GeneralFetalPath	No recorded effect
Difenoconazole	;DEV_Rat_General_FetalWeightRedu	;DEV_Rabbit_General_FetalWe
Thiodicarb	;DEV_Rat_General_FetalWeightRedu	No recorded effect

3. Virtual Embryo

in silico reconstruction



Multi-cellular models



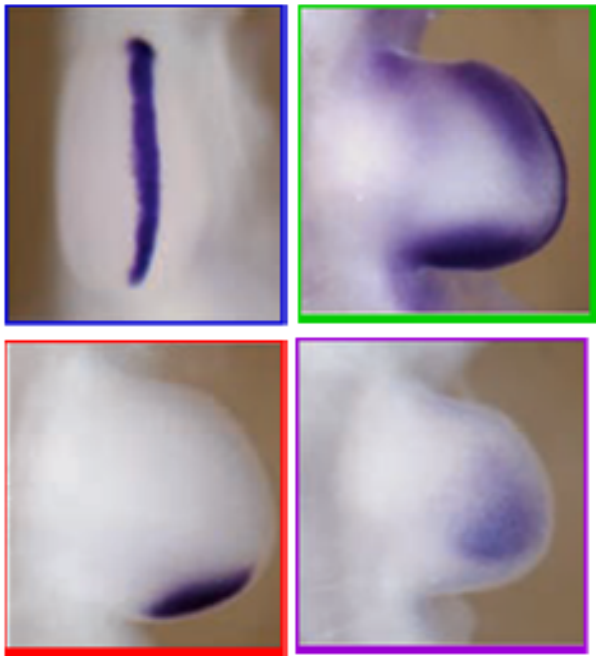
hits to skeletal_APP

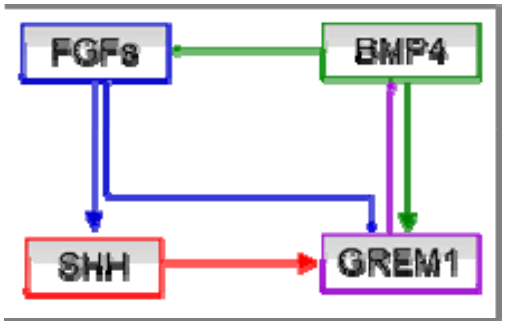
APPENDICULAR SKELETON
NVS_GPCR_hPY2
ATG_AP_1_CIS
ATG_ERRg_TRANS
BSK_KF3CT_TIMP2_up
BSK_LPS_Eselectin_up
BSK_LPS_IL8_up
BSK_SAg_CD40_up
MESC_CellUp
NVS_ENZ_hAbl_Activator
NVS_ENZ_hBTK_Activator
NVS_ENZ_hFyn
NVS_ENZ_hFyn_Activator
NVS_ENZ_hGSK3b
NVS_ENZ_hPP2Ca_Activator
NVS_ENZ_hPTPLAR
NVS_ENZ_hPTPMU
NVS_ENZ_hPTPPEST
NVS_ENZ_rMAOAC
NVS_GPCR_gBK2
NVS_GPCR_hAdra2A
NVS_NR_hPPARa
NVS_NR_hRAR
PS_Gene_ABL1
PS_Gene_ADRA2A
PS_Gene_Bdkrb2_Cavia_porcellus
PS_Gene_BTK
PS_Gene_ESRRG
PS_Gene_FYN
PS_Gene_GSK3B
PS_Gene_Maoa_Rattus_norvegicus
PS_Gene_P2RY1
PS_Gene_PPM1A
PS_Gene_PTPN12
PS_Gene_PTPRF
PS_Gene_PTPRM
PS_Ingenuity_0_cAMP_mediated_Signaling
PS_Ingenuity_0_Caveolar_mediated_Endocytosis
PS_Ingenuity_0_Fcgamma_Receptor_mediated_Phagocytosis_in_Macrophages_and_Monocytes
PS_Ingenuity_0_G_Protein_Coupled_Receptor_Signaling
PS_Ingenuity_0_Hypoxia_Signaling_in_the_Cardiovascular_System
PS_Ingenuity_0_PDGF_Signaling
PS_Ingenuity_0_T_Cell_Receptor_Signaling
PS_Ingenuity_0_Wntbeta_catenin_Signaling
PS_Ingenuity_a_Adrenergic_Signaling
PS_Ingenuity_Synaptic_Long_Term_Depression
PS_KEGG_0_Axon_guidance
PS_KEGG_0_B_cell_receptor_signaling_pathway
PS_KEGG_0_Fc_epsilon_RI_signaling_pathway
PS_KEGG_0_Pancreatic_cancer
PS_KEGG_0_VEGF_signaling_pathway
PS_KEGG_Axon_guidance
PS_KEGG_Long_term_depression
PS_PathwayCommons_0_CELL_MAP_Alpha6Beta4Integrin
PS_PathwayCommons_0_CELL_MAP_KitReceptor
PS_PathwayCommons_0_NCI_NATURE_Angiopoietin_receptor_Tie2_mediated_signaling
PS_PathwayCommons_0_NCI_NATURE_Aurora_A_signaling
PS_PathwayCommons_0_NCI_NATURE_Class_I_PI3K_signaling_events_mediated_by_Akt
PS_PathwayCommons_0_NCI_NATURE_Fc_epsilon_receptor_I_signaling_in_mast_cells
PS_PathwayCommons_0_NCI_NATURE_PDGFR_beta_signaling_pathway
PS_PathwayCommons_0_NCI_NATURE_S1P1_pathway

*Modeled in www.**CompuCell3d**.org environment*

N Poplawski (chick limb) → M Rountree (mouse limb)

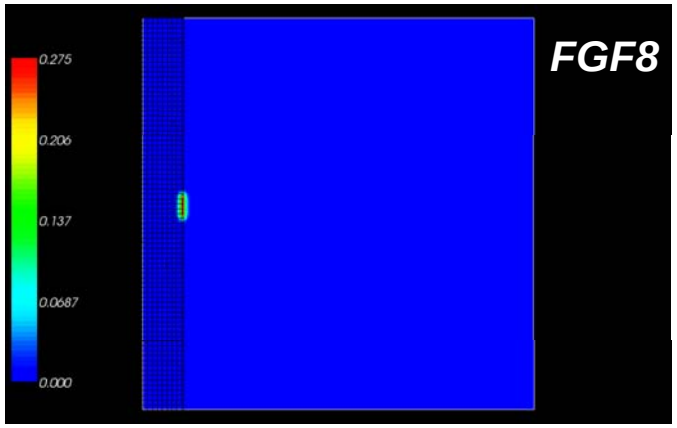
Cell Signaling Networks

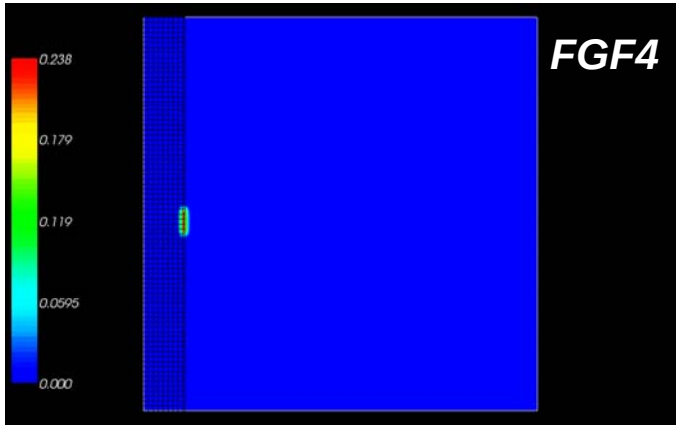




Gene network for limb outgrowth

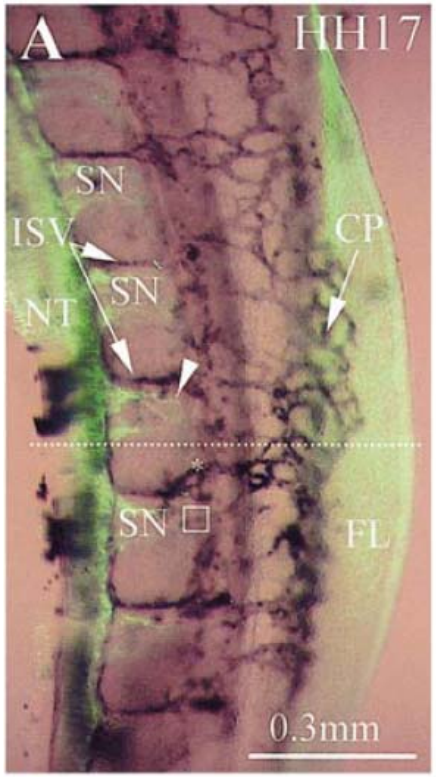
SOURCE: Benazet et al. (2009) Science 323:1050
Mackem & Lewandoski (2009) Science Signaling 2:pe33

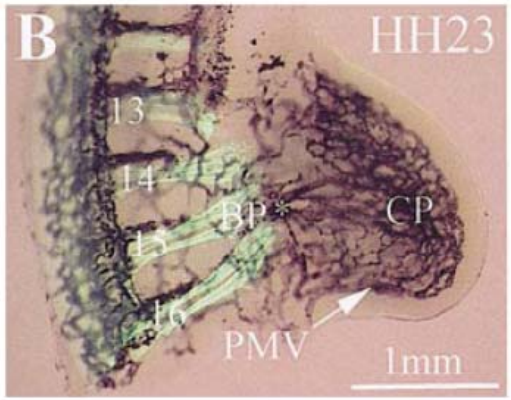




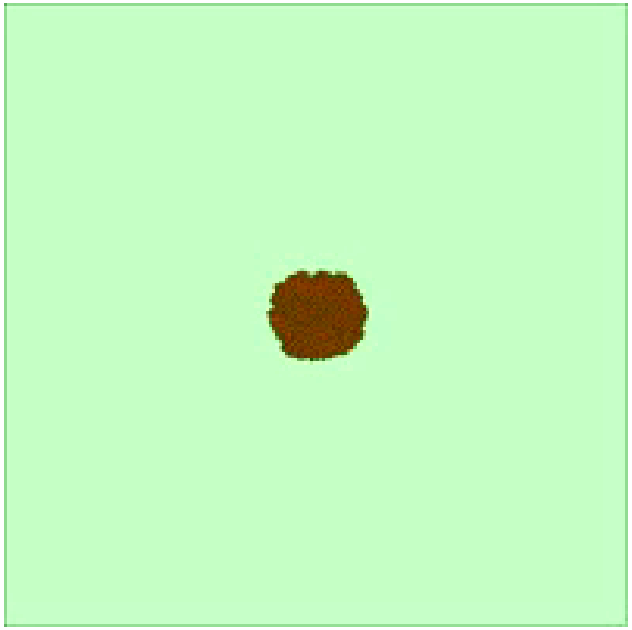
Multicellular model of FGF signaling built in CC3D

Vascular development





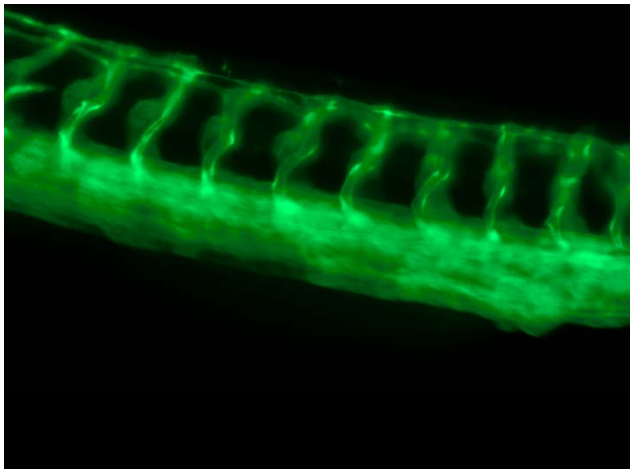
Bates, Taylor, and Newgreen (2002) The Pattern of Neurovascular Development in the Forelimb of the Quail Embryo. Dev Bio 249:300-320



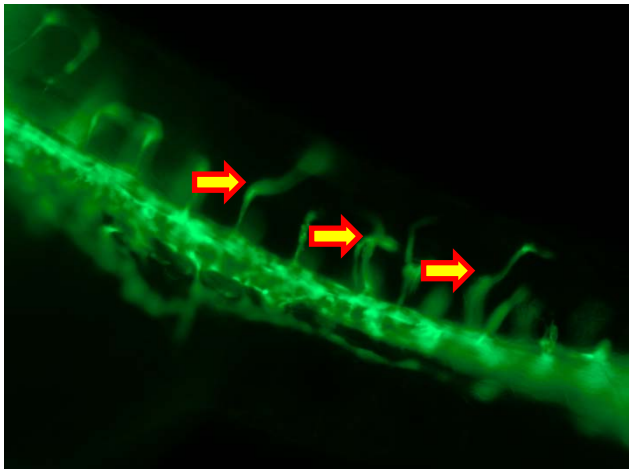
Merks, Perryn, Shirinifard, and Glazier (2008) Contact-Inhibited Chemotaxis in De Novo and Sprouting Blood-Vessel Growth. PLoS Comput Biol 4(9): e1000163

Validation:
zebrafish vascular development

72 h control (20x)



72 h treated (20x)



intersomitic vessel development monitored via Fli1 VEGFR transgene expression

SOURCE: M Bondesson & C McCollum, TIVS – University of Houston

Summary

- ToxCast™ is a resource to compile *in vitro* signatures into computational models that are diagnostic of *in vivo* toxicity
- toxicity in an intact organism results from numerous complex and inter-related events at a multi-cellular scale
- systems-level models that recapitulate *in vivo* biology can be used to assess the plausibility of diagnostic signatures
- multicellular 'virtual tissue' models can help bridge the gap between *in vitro* profiling and *in vivo* response

Credits

Virtual Embryo (NCCT)

Tom Knudsen
Amar Singh (LHM)
Michael Rountree (SSC)
Richard Spencer (EMVL)
Rob DeWoskin (NCEA)
Nikal Keinstreuer
Nisha Sipes

Virtual Embryo (NHEERL)

Sid Hunter
Stephanie Padilla
Kelly Chandler

Virtual Liver (NCCT)

Imran Shah
John Wambaugh
Woody Setzer
John Jack

Indiana University (CC3D)

Jim Glazier
Niko Poplawski
Maciej Swat
Abbas Shirinifard
Roland Merks

Texas-Indiana Virtual STAR Center (NCER)

Maria Bondesson (U Houston)
Jan-Ake Gustafsson (U Houston)
Don Fox (U Houston)
Richard Finnell (Texas A&M)
Jim Glazier (Indiana U)

ToxCast™ (NCCT)

Bob Kavlock
David Dix
Richard Judson
Keith Houck
Matt Martin
David Reif
Holly Mortensen

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