

Embryonic Vascular Disruption and Adverse Prenatal Outcomes

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Vascular Developmental

Processes

- endothelial proliferation & cell migration
 - growth factors
 - chemokine signaling
- extracellular matrix degradation
 - plasminogen activating system
 - matrix metalloproteinases
- neovascular stabilization
 - Ang/Tie2 signaling
 - vascular remodeling

Virtual Tissues-Knowledge Base (VT-KB): ~100 distinct ToxCastDB assay targets map to key systems in vascular development



ToxCastDB: 700+ HTS Assays



(http://actor.epa.gov/actor/faces/ToxCastDB)

ToxCastDB



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AC50 concentration producing a 50% change LEC lowest effect concentration

ToxCastDB

You are here: EPA Home , National Center for Computational Toxicology , ToxCastDE , Assay ACTWR TristleTOR ToxCastDE ExpectantDE DISTIN

> Novascreen NV5_ENZ_NVEGFR2 Novascreen Human VEGFR2 Human VEGFR2 Fluorescein-labeled peptide

320 1 Homo sapiens

Home ¹ Basic Info ¹ Data Collection List ¹ Chemical List ¹ General Second with Assays ¹ Help

Assay:	Novascreen	Human VEGFR2
Assay Id:		9
Source		N
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Name		N
Description		14
Number of Sub	stances	3
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Parameters Value Parameter CATALOG NUMBER 200-0768 ASSAY CATEGORY Enzyme Inhibition ASSAY CATEGORY In vitro (Biochemical) ASSAY TARGET VEGFR2 ASSAY TARGET FAMILY Kinsse ASSAY TARGET SOURCE Recombinant ASSAY TARGET SOURCE TYPE amino acid 805 to 1356 ASSAY GENE ID 3791 ASSAY GENE NAME KDR ASSAY REFERENCE COMPOUND Staurosporine KINASE ASSAY NOTE ASSAY SUBSTRATE NAME receptor tyrosine kinase ASSAY ATP CONCENTRATION (M) NCCT_v2 ASSAY ENZYME AFFINITY ATP KM (M) Fluorescein-labeled peptide ASSAY LIGAND NAME 1.50E-05 ASSAY LIGAND CONCENTRATION (M) 1 20E-05 ASSAY BMAX Fluorescein -peptide + ATP --> fluorescein -phosphopeptide + ADP

	Data	
Name	CASRN	NVS_ENZ_hVEGFR2 (uM)
Mancozeb	8018-01-7	5.9
Maneb	12427-38-2	31.0
Metiram-zinc	9006-42-2	45.0
Oxytetracycline dihydrate	6153-64-6	19.0



- Gene Ontology (GO) and Mammalian Phenotype (MP) browsers of MGI database (<u>http://www.informatics.jax.org/</u>) for neovascularization:
 - abnormal vasculogenesis [MP:0001622; 72 genotypes, 73 annotations]
 - abnormal angiogenesis [MP:0000260; 610 genotypes, 894 annotations]
- 65 genes with roles in vasculogenesis or angiogenesis linked to ToxCast assays, 50 had evidence of abnormal embryonic vascular development in MGI

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Overlap between ToxCast assay targets and abnormal vascular phenotypes from genetic mouse models.

ToxCast Gene Target *	MP Annotated Term	ToxCast Assays			
AHR	patent ductus venosus, abnormal vascular regression	ATG_Ahr_CIS, NCGC_AhR,			
BMPR2	decreased angiogenesis	ATG_BRE_CIS			
CASP8	abnormal vitelline vasculature morphology	NVS_ENZ_hCASP8			
CCL2	decreased angiogenesis, abnormal physiological <u>neovascularization</u> , choroidal neovascularization	BSK_3C_MCP1, BSK_4H_MCP1, BSK_KF3CT_MCP1, BSK_LPS_MCP1, BSK_SAg_MCP1, BSK_SM3C_MCP1			
CEBPB*	abnormal vasculogenesis, absent organized vascular network	ATG_C_EBP_CIS, ATG_CRE_CIS			

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diverse biological targets and build	Disruption of e	Review Disruption of embryonic vascular development in predictive toxicology [†]					SEARCH			
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Adverse Outcome Pathway Framework



Established mechanistic linkage with quantitative or semi-quantitative data



Predictive model linkages based on quantitative concentration-response data



Test the pVDC signature



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Chemical selection: Test the pVDC signature



Validation of vascular disruption AOP by orthogonal assays: in vitro, in silico, and in situ

In vitro (HUVEC) Noguchi et al. 2005, Bioorg Med Chem Lett.

In silico (virtual tissue) Kleinstreuer et al (2013) PLoS Comp Biol 9(4): e1002996

In situ (Aortic explant) Carney & Ellis-Hutchings, Dow Chemical Co. (manuscript in prep)



5HPP-33 exposure disrupts angiogenesis in vitro, in silico, and in situ

Virtual Tissues & Human Cell Based Tubulogenesis



SOURCE: FICAM (T Heinonen and R Sarkanen)¹²



Zebrafish embryogenesis: A Quantitative AOP Model?

- a biologically complex system
 vascular developmental toxicity
- conserved pathways
- 75% of genes have human homologs
- embryo is transparent
- amenable to quantitative imaging
- transgenic reporter lines
- map vasculature across space-time
- rapid and scalable platform
 - amenable to automation and HTS







SOURCE: Tamara Tal, EPA/NHEERL-ISTD

Zebrafish trunk vessel assay Screening strategy



- Quarter-log spacing
- 8 concentrations
- n=3, 2 embryos/rep
- PTK787 positive control (4 μM)

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Impaired angiogenesis in larvae exposed to PTK787 (VEGFR2 inhibitor)



Predominant CVP phenotype: Haloperidol



SOURCE: T. Tal, EPA/NHEERL-ISTD

Cranial vascular phenotype: Fluazinam



0.4% DMSO

0.47 µM Fluazinam

Vascular Toxicity Results (Red: hit in at least one assay, Green: no hit)



SUMMARY

- Quantitative AOPs using HTS dose response data allow for hypothesis generation, modeling and testing of MIEs and cellular interactions that may lead to toxicities.
- AOP validation is facilitated via orthogonal assays, small model organisms such as zebrafish, and other scientifically relevant information.
- Our group has generated and evaluated a vascular development screening tool utilizing phenotypic endpoints.
- Preliminary data shows that chemical rankings are generally well correlated among the predictive signature, zebrafish overt toxicity and *in vitro* tubulogenesis assays.
- Validated AOPs enable chemical prioritization, high throughput risk assessments, and probabilistic frameworks.

Future directions: Leveraging diverse data streams to improve the developmental vascular toxicity signature



- 1. Are all of the assays relevant and how should they be weighted?
- 2. What non-ToxCast targets are needed?
- 3. Should other ToxCast assays be included?
- 4. Are we taking full advantage of the zebrafish model?
- 5. What other orthogonal assays should be used to test pVDC predictions?

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Questions?

Functional consequence of vascular disruption during development

