

Modeling Nuclear Receptor Mediated Pathways in Liver Cancer

Society of Toxicology
March 9, 2010
Virtual Liver Project (v-Liver™)
I Shah, DC Wolf, K Houck, R Judson, J Jack, J Wambaugh, MT Martin, C Corton

UNITED STATES ENVIRONMENTAL PROTECTION AGENCY COMPUTATIONAL TOXICOLOGY

This work was reviewed by EPA and approved for publication but does not necessarily reflect official agency policy.

Office of Research and Development National Center for Computational Toxicology



Predictive Models of Liver Cancer

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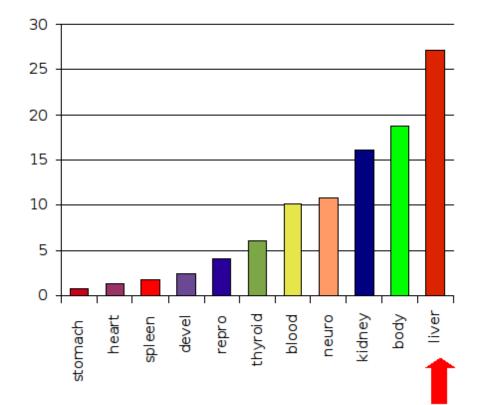


- Thousands of environmental chemicals
- Insufficient information on human risk
- Rodent testing infeasible / uncertain results
- Need other decision support tools
- Develop proof-of-concept using
 - Existing in vivo data
 - New in vitro data
 - Predictive in silico systems



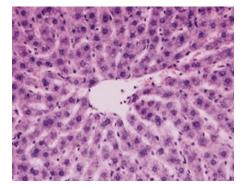
- Liver primary organ for detoxification
- Frequent site of adverse effects in rodents
- Human relevance of some effect uncertain
- Large amount of available data: -omics, histopathology, etc.

EPA Integrated Risk Assessment System (IRIS) (Oral RfDs, Non-cancer endpoints)



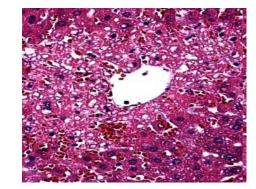


Hydropic Swelling



Hypertrophy

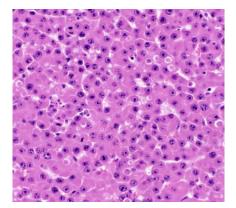


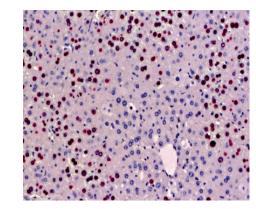


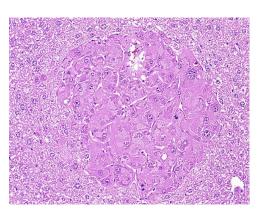
Apoptosis

Regenerative Prol.

Altered Hep. Focus





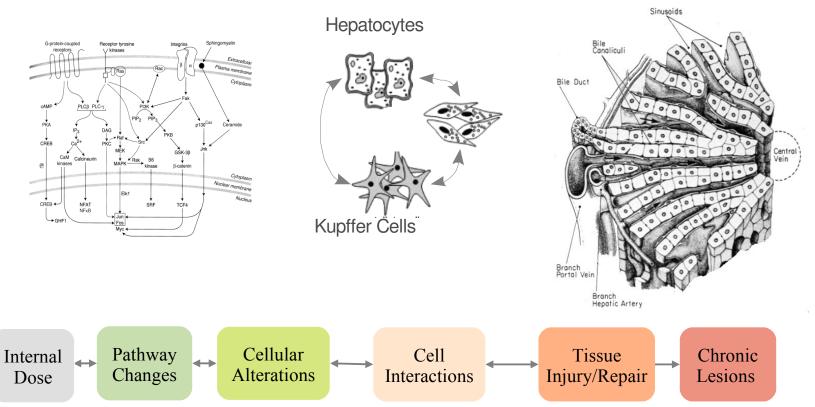


Images courtesy of Douglas C. Wolf, EPA Fallsehr, et. al. World J Gastroenterol 2005 March 7;11(9):1303-1306 Jean-Paul Duong Van Huyen, et. al. Modern Pathology (2006) 19, 1277-1288.0 Uskoković-Marković, et. al. J Pharm Pharmaceut Sci 10(3):340-349, 2007



Systems View of Lesion Formation

Liver Lobule

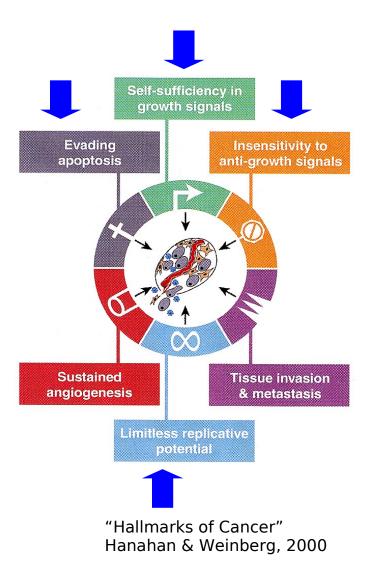




Multiple MOAs: Mutagen, mitogen, cytotox Nuclear Receptor (NR) activation relevant in rodent hepatocarcinogenesis

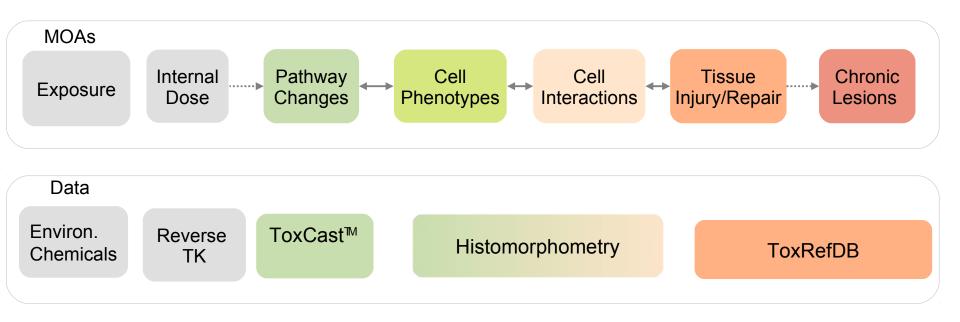
Agency

Can we extrapolate events to humans? NR-activation Changes in apoptosis/proliferation Cancer lesion progression





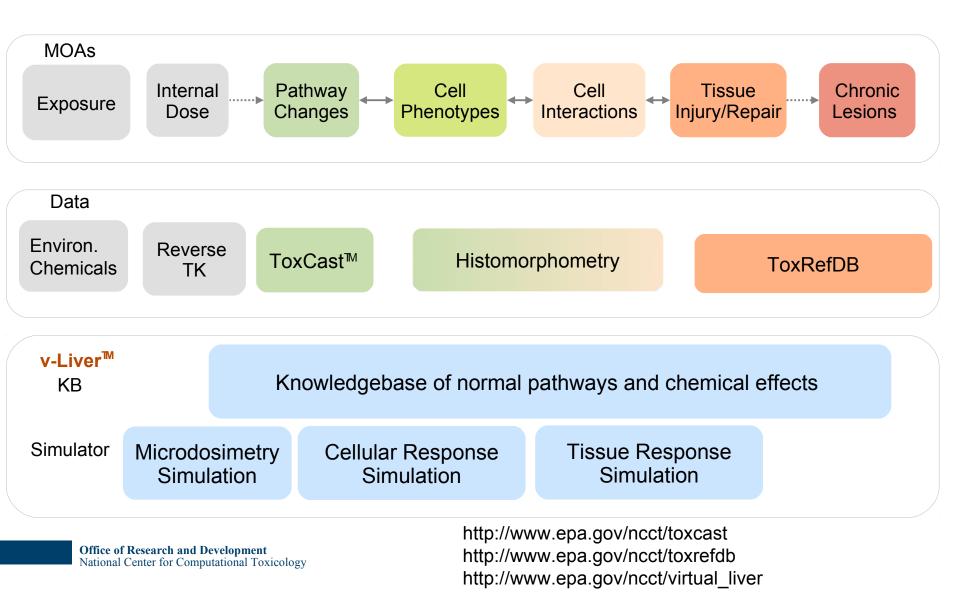
Virtual Liver Overview



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

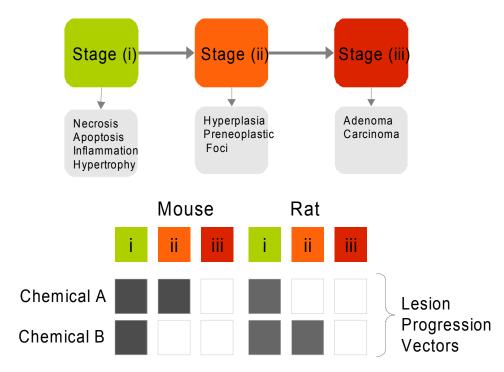


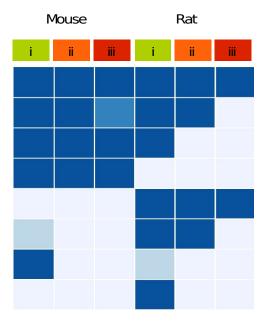
Virtual Liver Overview





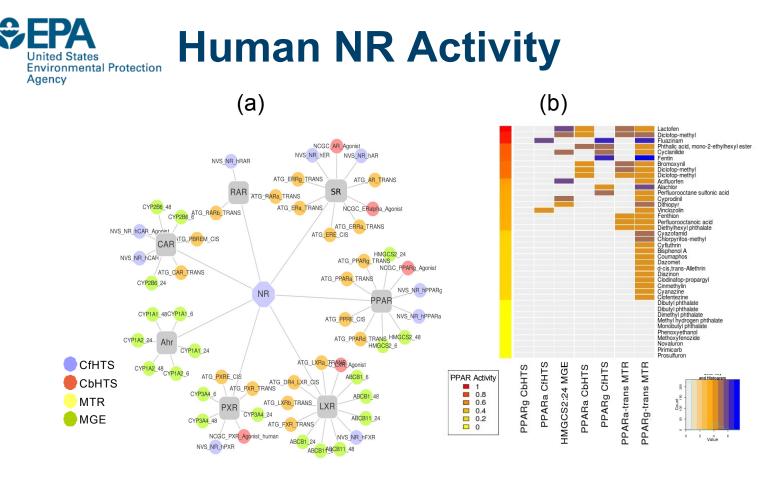
Selecting Chemicals Using Rodent Histopathology





ToxRefDB

Office of Research and Development National Center for Computational Toxicology Shah, *et al.* (submitted) http://www.epa.gov/ncct/toxre fdb



ToxCast: A combination of assays: cell free/cell-based NR bioactivity, multiplexed transcription reporters, multiplexed gene expression Find the "aggregate" activity of each chemical across NRs e.g. PPARs

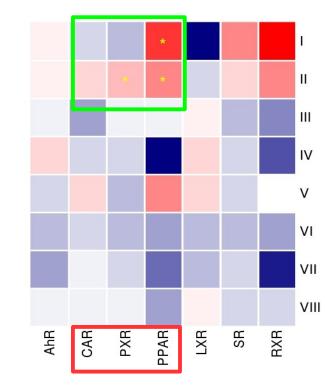
Shah, et al. (submitted)

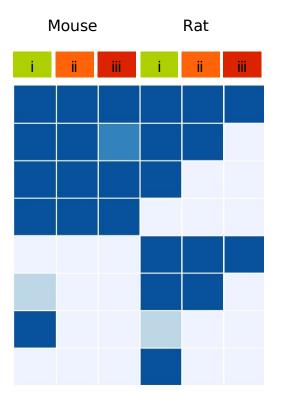


Aggregate NR Activities: Examples

AhR	CAR	PXR	PPAR	LXR	SR	RXR	
							(A, VI) Tetraconazole
							(A, IV) Triadimenol
							(A, IV) Triadimefon
							(A,III) Propiconazole
							(A,VIII) Hexaconazole
							(A, ?) Diniconazole
							(A,VII) Triticonazole
							(D,VIII) Flusilazole
							(E,III) Cyproconazole
							(F,VII) Fenbuconazole
							(F,VII) Myclobutanil
							(F,III) Difenoconazole
							(A, II) Triflumizole
							(A,III) Iprodione
							(B, I) Imazalil
							(B, ?) Cyazofamid
							(D,III) Prochloraz
							(E, II) Fenamidone
							(B, ?) Phthalic acid, mono-2-ethylhexyl
							(D, I) Diethylhexyl phthalate
							(F, ?) Dibutyl phthalate
							(G, ?) Monobutyl phthalate
							(G, ?) Dimethyl phthalate
							(G, ?) Methyl hydrogen phthalate



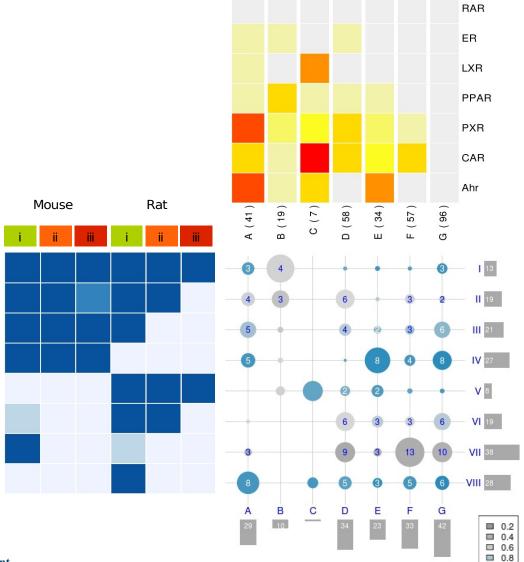




NR FC ■ 4.0 ■ 2.0 □ 1.0 ■ 0.5 ■ 0.2

Shah, et al. (submitted)

NR activity vs. Rodent Cancer



United States Environmental Protection Agency

> Office of Research and Development National Center for Computational Toxicology

Shah, et al. (submitted)

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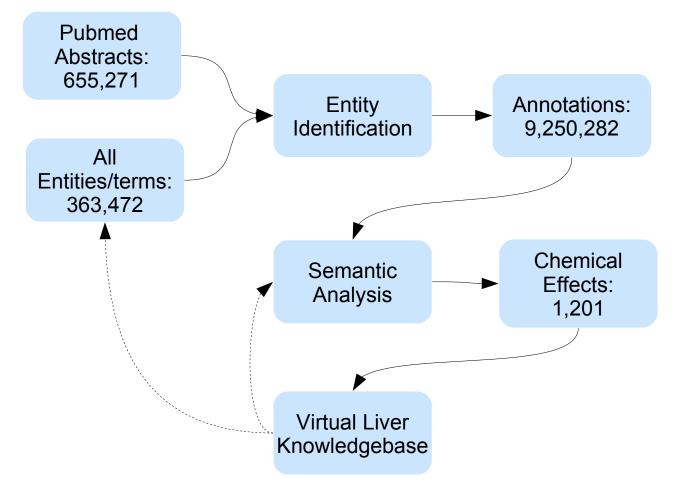


Relating NR Activity and Pathways

- Need to mine information from literature and public domain data
- Use natural language processing (NLP) tools
- Semi-automated curation of chemical-induced effects
- Store all information in v-Liver Knowledgebase



Literature Curation Overview





Semantic Analysis & Curation

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🔲 vLiverKB: SAC File Help Entity search phenobar loading entities & abstract for PMID:11146218 ... Carcinogenic potential of cooked food mutagens (IQ and MeIQ) in Wistar rats after Filter None Filter tissue-event Epoprostenol sodium, a prostaglandin I2, lacks tumor promoting effects in a mediu Absence of liver tumor promoting effects of annatto extract (norbixin), a natural car kegg Phenobarbital chemical NΑ 11.64 1 tissue-event vl eosinophilic focus 14.88 2 tissue-event chemical kegg Luminal NA vl hepatic carcinogenesis kegg Mebaral NA chemical 16.73 3 tissue-event vl tumour initiation NA chemical kegg Luminal sodium 16.75 1 tissue-event vl tumour promotion chemical drugbank Phenobarbitone NΑ 16.82 4 tissue-event vl neoplasm prot-rn uniprot Aldehyde dehydrogenase, cytosolic 1 Aldehyde dehydrogenase, 17.30 1 tissue-event vl degeneration prot-mm uniprot Aldehyde dehydrogenase, cytosolic 1 Aldehyde dehydrogenas 17.54 1 tissue-event vl edema mesh-organic-chemical mesh Mephobarbital A barbiturate that is metabolized 19.61 3 tissue-event vl cirrhosis mesh-organic-chemical mesh Phenobarbital A barbituric acid derivative that a vl hepatocellular carcinoma 19.74 2 tissue-event mesh-organic-chemical mesh Primidone An antiepileptic agent related to the 19.78 1 tissue-event vl proliferation 19.93 4 tissue-event vl carcinoma Entities Chunks Terms TrmMatches SemMatch ChkGrm PatSem PMID Analyze Entity? Chunks Entities Type Term TITLE: Epoprostenol sodium, a prostaglandin I2, lacks tumor promoting 🕁 sent-001 effects in a medium-term liver carcinogenesis bioassay in rats. NPHR Potential/JJ modifying/NN effects/NNS ch Date: 2001-01-30 00:00:00 PREP of/IN ch PMID: 11146218 ?|chemi epoprostenol/NN sodium/NN administration/NN ch Potential modifying effects of epoprostenol sodium administration on liver carcinogenesis PREP on/IN ch were investigated in male F344/DuCri rats initially treated with N-nitrosodiethylamine (DEN). Two weeks after a single dose of DEN (200 mg/kg, intraperitoneally), rats daily ? Itissue liver carcinogenesis er received subcutaneously epoprostenol sodium at doses of 0, 1, 10 and 100 microg/kg, VERB were/VBD investigated/VBN ch or were fed phenobarbital sodium (PB) at a dietary level of 500 parts per million (ppm) PREP in/IN ch as positive control for 6 weeks. All animals were subjected to partial hepatectomy at week 3, and were killed at week 8. Prominent flushing of extremis and signs of ?[mesh-male/]] F344/DuCrj/NN rats/NNS ch behavioural depression occurred after injection and lasted for 1 h in rats given 100 VERB initially/RB treated/VBN ch

microg/kg epoprostenol sodium. Such clinical signs were slight in rats treated with 10 microg/kg, but not observed with 1 microg/kg. Marked decrease in body weight gain was noted in rats given 100 microg/kg. Statistically significant changes in relative liver weights were not found in any group given the test chemical. Epoprostenol sodium did not significantly increase the quantitative values for glutathione S-transferase placental form (GST-P) positive liver cell foci observed after DEN initiation, in clear contrast to the positive control. The results thus demonstrate that epoprostenol sodium lacks modifying

Shah	&	Haugh
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PREP

> sent-002

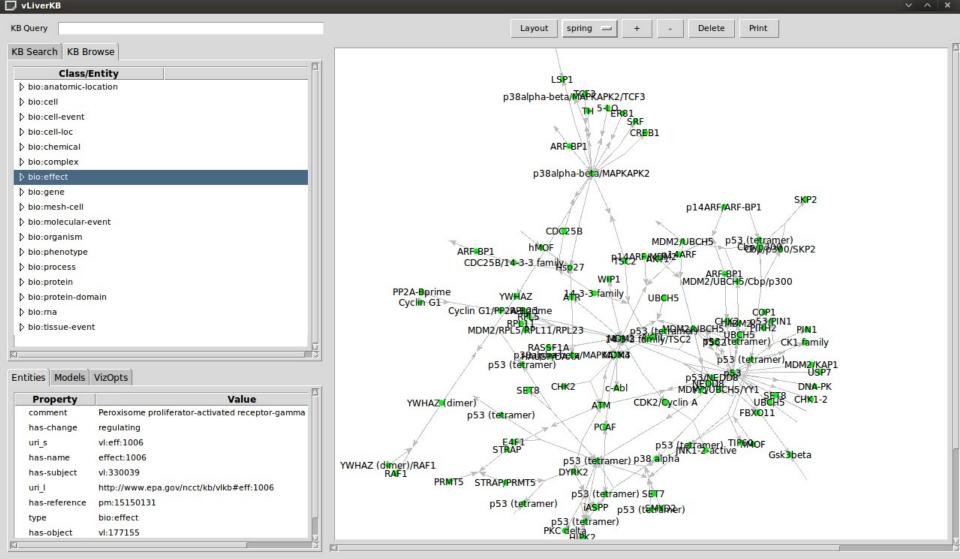
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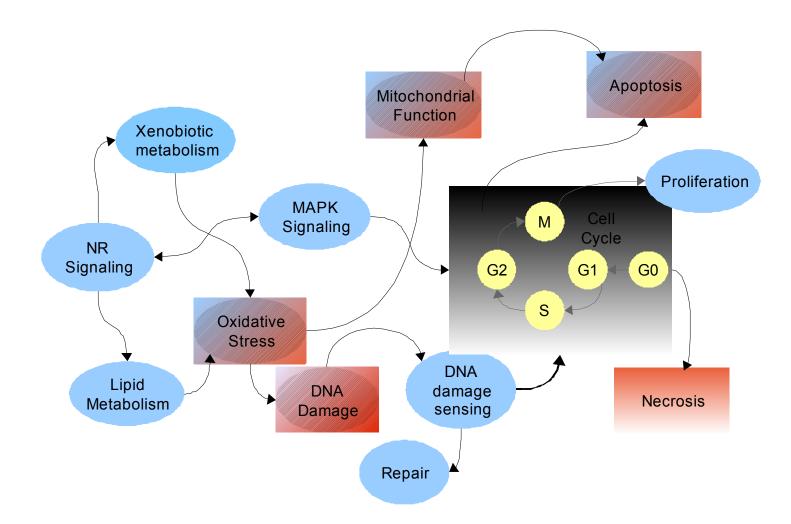
Virtual Liver Knowledgebase



Office of Research and Development National Center for Computational Toxicology

Environmental Protection

Knowledgebase: Cellular Processes



United States

Agency

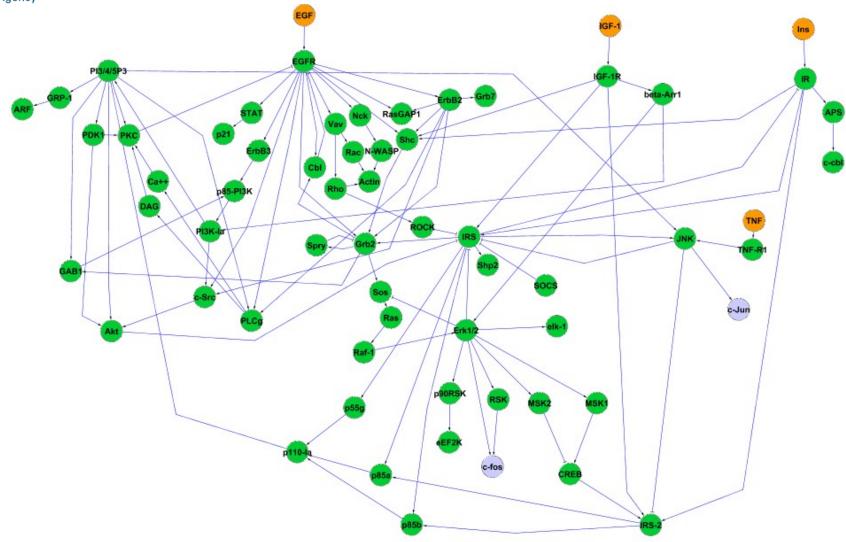
Environmental Protection



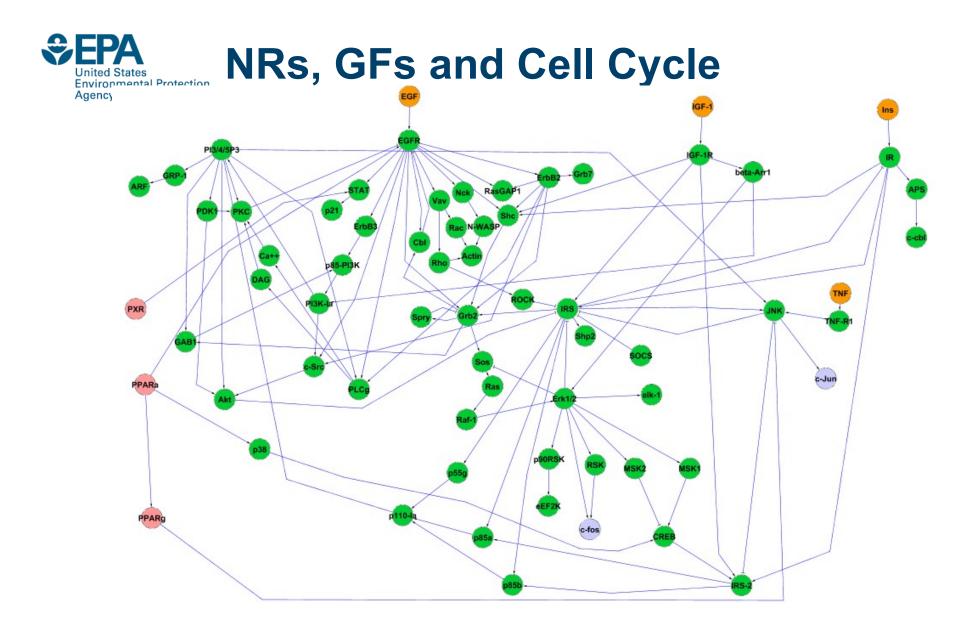
- Use KB to develop systems model of NRmediated pathways
- E.g. synthesize evidence growth factor (GF) induced cell cycle changes (G1/S-transition)
- Evaluate potential crosstalk between NR and GF-signaling

Growth Factor Crosstalk: Cell Cycle



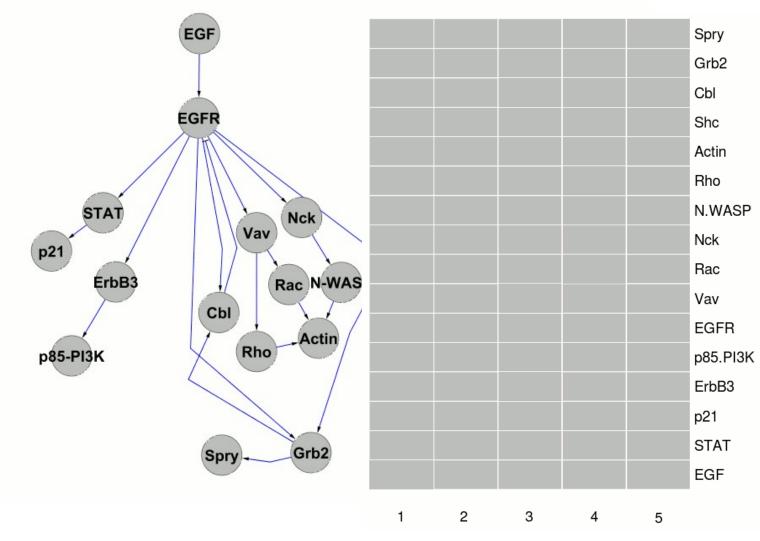


J. Jack



Simulating Signal Propagation





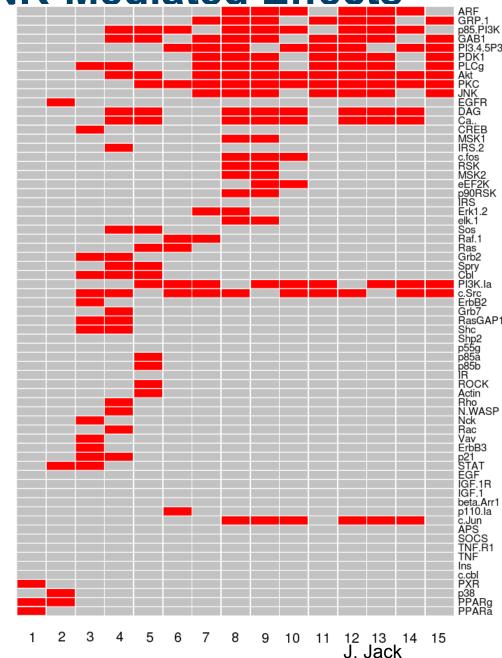
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J. Jack



Simulate NR-Mediated Effects

- Transient Activation of PPARa, PPARg, and PXR
- Effects:
 - cFos activation (t=8)
 - cJun activation (t=8)
 - Erk1/2 activity (t=7)
 - p21 activation (t=3)
 - Akt activation (t=7)

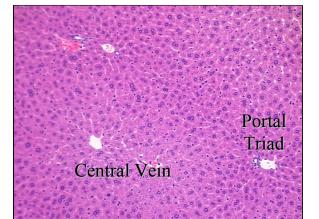




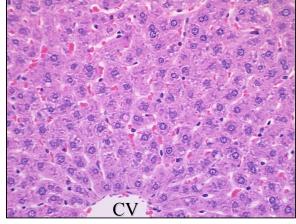
Quantitative Tissue Modeling



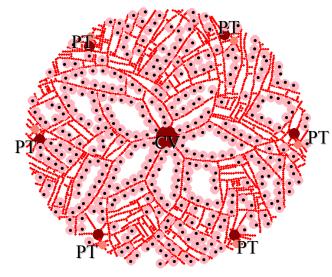
Dose-Response for a Virtual Lobule



The classic lobule consists of a single central vein fed venous and arterial blood via multiple portal triads



Blood flows through a network of sinusoids, supplying and exposing hepatocytes



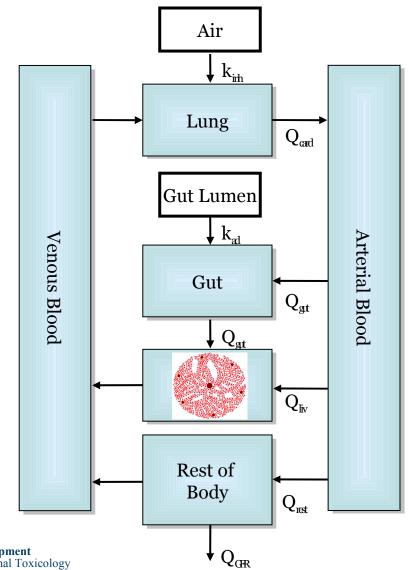
Synthetic lobule sufficiently complex to determine that approach would work with actual lobule morphology (e.g. Drasdo et al.)

Wambaugh & Shah (submitted)

photo credits: Rockett et al₂₆(2006) <u>Reproductive Toxicology</u> **22** 647-658



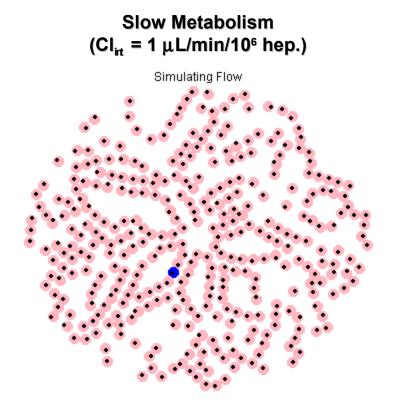
Integration with Pharmacokinetic Models



- Our lobule module is directly integrated with a pharmacokinetic model.
- Determines specific amount of chemical delivered to any given hepatocyte in μmol.
- Suitable for endogenous and xenobiotic compounds.



Simulating Microdosimetry and Effects



Concentration Above Threshold Below Cytotoxicity Threshold

- **H**^o (Normal/Quiescent)
- H^{atht} (Stressed/Adaptive)
- H^{ij} (Stressed/Injured)
- H^{nc} (Necrotic)
- H^{pd} (Proliferative)
- Happer (Apoptotic)

Wambaugh & Shah (submitted)



- Goal: NR-mediated cellular pathways, alterations and lesions
- Integrative computational and experimental paradigm is vital for success
- Next steps: *short-term in vivo* study design and assays evaluating *in vitro* data, MOA/dose-response



Multi-disciplinary Team: Cross-EPA/ORD & External Collaborations

