Use of Toxico-omics to Discriminate Conazoles and Related Chemicals from Phenobarbital for Human Relevancy Determinations in Cancer Risk Assessments

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LTG 1 Poster 02

Science Questions

•Can toxico-omics discriminate between conazoles or related nongenotoxic mouse liver carcinogens, and phenobarbital (PB), the prototypical nongenotoxic mouse liver carcinogen?

•Can these "omic"approaches be used in the human relevancy framework for assessing the validity of using the phenobarbital MOA in the cancer risk assessments of these agents?

Research Goals

•To define a general toxico-omics approach that can discriminate PB from other mouse liver tumorigens: Two conazoles were examined, propiconazole (Pro) and triadimefon (Tri).

•To integrate transcriptomic, proteomic, and metabolomic data to give a more complete picture of the differences and similarities between PB and other nongenotoxic mouse liver carcinogens.

•We posit that the transcriptional profiles of tissues exposed to toxic chemicals inherently contain their mechanisms of toxicity. If the transcriptional profiles induced by different chemical treatments are dramatically different then their mechanisms of toxicity/MOA would also differ.

Summary and Conclusions

We have devised a series of bioinformatic tools that can discriminate between mouse liver tumorigenic conazoles (Pro, Tri) and PB. The distinguishing profiles are based on mapping genes to known functional gene lists (e.g. cell cycle); to fixed pathways (e.g. canonical pathways); and to dynamic networks and central hubs.

Targeted comparisons of cell cycle genes, transcription factors, Gene-Go pathways, networks, and their central hubs that control groups of genes revealed significant differences between PB and the conazoles. Moreover, similarities between conazoles were observed.

Genes expressed by PB or conazoles were compared to those genes associated with human hepatocellular cancer. Conazole exposure was associated with a different complement of genes compared to PB.

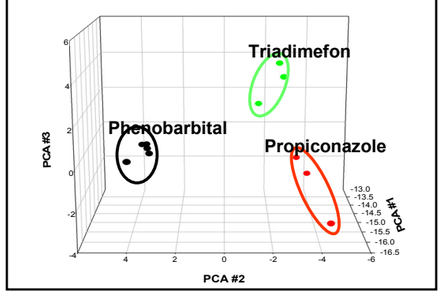
We conclude that although PB and conazoles induce mouse liver tumors, and each exhibits similar phenotypic responses, their transcriptional profiles are different, and their mechanisms of tumorigenic action/MOA are highly likely to differ.

Our transcriptomic data on conazoles and PB are richer in detail and substance compared to the presently accepted observational criteria for defining the PB MOA. If validated, they could serve to replace or augment the existing method of discrimination.

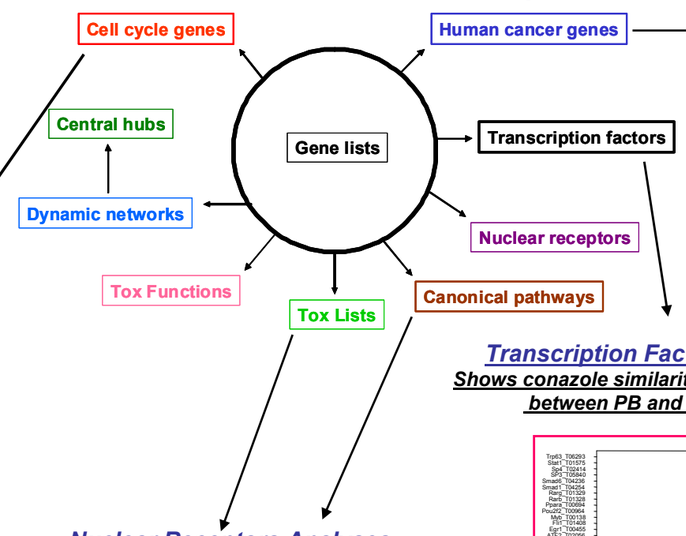
Results

Global Analyses

Differential gene expression in mouse liver shows similarity between conazoles (Tri and Pro) and dissimilarity to PB

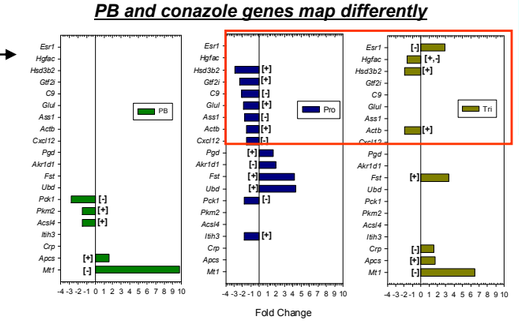


Genomic data was evaluated on multiple levels



Human Hepatocarcinoma Associated Genes

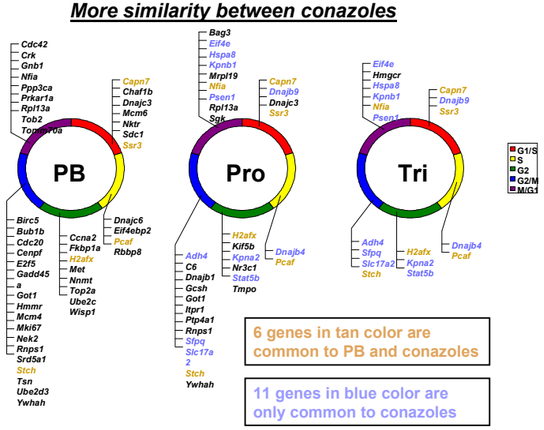
PB and conazole genes map differently



Legend
[+] overexpressed in human HCC
[-] underexpressed in human HCC

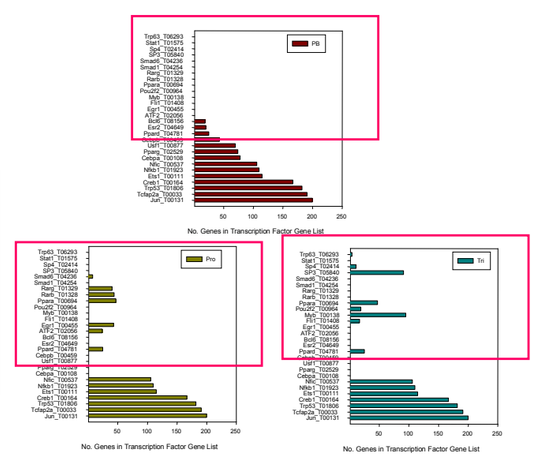
Cell Cycle Gene Analyses

Major differences between PB and conazoles
More similarity between conazoles



Transcription Factor Analysis

Shows conazole similarity & discriminates between PB and conazoles



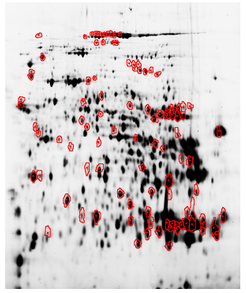
Nuclear Receptors Analyses

Tox Lists and Canonical Pathways

PB Treatment
CAR/RXR Activation
PXR/RXR Activation
FXR/RXR Activation
PPARα/RXR Activation
PPARα/RXRα Activation
RAR Activation

Pro & Tri Treatment
CAR/RXR Activation
PXR/RXR Activation
FXR/RXR Activation
PPARα/RXR Activation
PPARα/RXRα Activation
RAR Activation
LXR/RXR Activation
TR/RXR Activation

Example of Proteomics Data



2D-DIGE of liver cytosol from mice treated with propiconazole. Red circles indicate differentially expressed proteins compared to control.

Impact and Outcomes

•These extremely important analyses have high relevance to OPP. They will use this information for future evaluations of conazoles and related classes of pesticides.

•This mechanistic information and these bioinformatic approaches will be used by OPP in the risk assessment process for pesticides that are mouse liver tumorigens and produce PB-like responses.

•The outcomes will be more scientifically-defensible approaches to determine whether the MOA of these pesticides are unlike PB, or like PB and thus relevant, or not relevant to humans.

Future Directions

The focus of the future studies will compare PB and tumorigenic conazoles on the proteome and metabolome levels.

Targeted proteomic studies using cell fractions (nuclear, cytoplasmic, and microsomal) will allow the identification of key proteins that discriminate between the different treatments.

Metabolomic analyses of livers and urines from mice will also be sought. This will lead to identification of the biomarkers that can be used to compare the Key Events/MOA of new chemicals with that of PB.

All of these approaches: transcriptomic, proteomic, and metabolomic will be integrated to give a more complete picture of the differences between PB and other nongenotoxic mouse liver carcinogens.

Researchers Involved

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