A New Approach Methodology for the Prediction of Tumorigenesis

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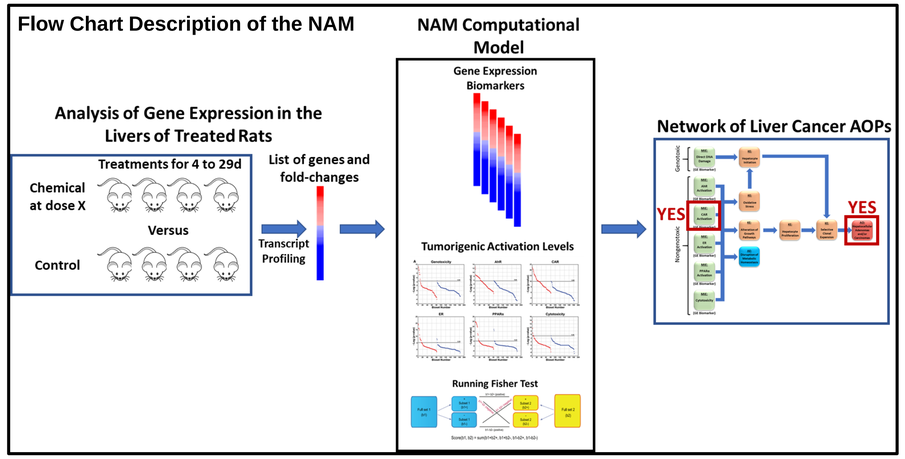
Abstract

Cancer is the 2nd leading cause of death in the U.S., putting tremendous pressure on the economy. Tens of thousands of chemicals on the market have not been adequately tested for cancer hazard. Testing using the "gold standard" 2-year cancer bioassay is not feasible to fill this data gap. Thus, new approach methodologies (NAMs) are necessary to assess potential carcinogenicity of uncharacterized chemicals. The present study shows how a NAM can be used to identify chemicals and their doses that could cause liver cancer in rats without having to conduct a 2-year bioassay. The NAM uses 6 previously established gene expression biomarkers and tumorigenic activation levels (TALs) to interpret transcript profiles derived from the livers of treated rats. The NAM can identify the chemical mode of action and (non)tumorigenic doses. While this NAM cannot completely replace rodent bioassays, this approach can be used to help determine if the 2-year bioassay is necessary, potentially reducing cost, time, and resources needed.



Methods

Rat Short-term Exposure Studies

- Male SD rats exposed once daily by oral gavage; liver, blood collected; liver RNA analyzed
  - 4-Day Study: 22 chemicals, 1 dose; Affymetrix platform
  - 5-Day Study: 16 chemicals, up to 10 doses; full genome BioSpyder TempO-Seq platform

Determination of Chemical Hepatocarcinogenicity

- Lhasa Carcinogenicity Potency Database and ToxRef database were used to annotate incidences of the following in 2-year cancer bioassays: hepatocellular carcinomas, adenomas; multiple liver tumor types; liver neoplastic nodules; trabecular hepatocellular carcinomas; hepatocellular cholangiocarcinomas
- Doses used in transcript profile studies were annotated for highest nontumorigenic dose and lowest tumorigenic dose (5% over baseline was considered tumorigenic)

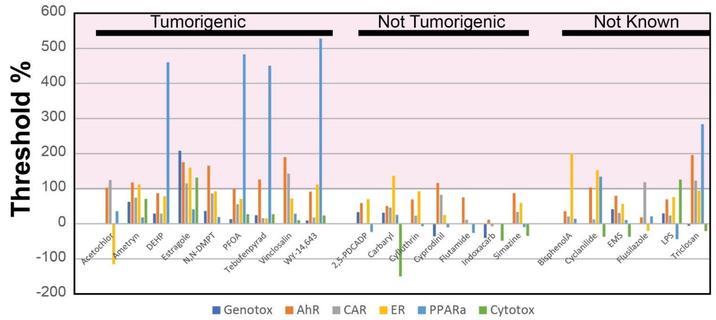
Comparison of Biomarkers to Gene Lists

- Gene lists created during rat studies were uploaded into BaseSpace Correlation Engine (BSCE) with 6 previously established gene expression biomarkers: AhR, CAR, PPARα, ER, cytotoxicity, genotoxicity
- BSCE ranked genes by absolute fold-change; the Running Fisher test compared biomarker gene ranks against gene ranks from the rat studies; correlations were made on the overlapping genes to obtain p-values converted to -Log(p-values)

Application of Biomarker Tumorigenic Activation Levels (TALs)

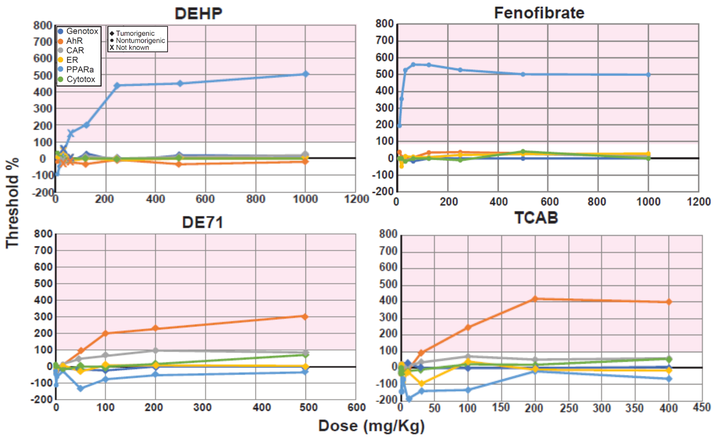
- Defined as highest -Log(p-values) that did not induce liver tumors from previous studies using a large genomic database derived from the livers of rats treated with ~130 chemicals (TG-GATES) or ~300 chemicals (DrugMatrix)
- Biomarker -Log(p-values) from the 2 studies were evaluated relative to the TALs to determine if short-term exposure to a chemical-dose pair exceeded the biomarker activation level

Biomarker tumorigenic activation levels identify tumorigenicity of chemical-dose pairs in a 4-day short-term exposure study



The 6 biomarkers were compared to the transcript profiles from short-term rat exposure studies. The -Log(p-value)s resulting from the correlation between the chemicals and the biomarkers were compared to the tumorigenic activation levels. The y axis represents the (biomarker -Log(p-value)/the tumorigenic threshold) x 100. Any -Log(p-value) >100% for any of the biomarkers is predicted to increase liver tumors in a chronic exposure.

Prediction of chemical doses that would increase liver tumors in rats in 5-day short-term exposure studies



Gene expression was evaluated using targeted RNA-seq (TempO-Seq), and the derived gene lists were compared to the 6 biomarkers using the Running Fisher test. The -Log(p-value)s from the correlation between the chemical-dose pair and the biomarkers were compared to the tumorigenic activation levels. The y axis represents the (biomarker -Log(p-value)/the tumorigenic threshold) x 100. Any -Log(p-value) >100% for any of the biomarkers is predicted to increase liver tumorigenicity in a chronic exposure. Note: only 4 of the 16 chemicals are shown.

Predictive accuracies derived using this NAM

Study	Unit of prediction	Tumorigenic activation level	Total Number of Biosets or Chemicals Examined	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV	Balanced Accuracy
Rat 4-day study	Chemical-Dose	TG-GATES	13	8	3	1	1	0.889	0.75	0.889	0.75	0.819
Rat 4-day study	Chemical-Dose	DrugMatrix	13	7	4	0	2	0.778	1	1	0.667	0.889
Rat 5-day Study	Chemical-Dose	TG-GATES	100	31	51	7	11	0.738	0.879	0.816	0.823	0.809
Rat 5-day Study	Chemical-Dose	DrugMatrix	100	22	56	2	20	0.524	0.966	0.917	0.737	0.745
Rat 5-day Study	Chemical	TG-GATES	16	11	3	2	0	1	0.6	0.846	1	0.8
Rat 5-day Study	Chemical	DrugMatrix	16	9	5	0	2	0.818	1	1	0.714	0.909

The TALs of the 6 biomarkers (derived using the TG-GATES and DrugMatrix studies) were used to predict hepatocellular adenomas and/or carcinomas. The balanced accuracy is up to 91%.

Summary

The NAM can be used in a number of testing conditions to accurately identify chemicals and their doses that would induce tumors in the livers of rats

- The NAM can predict tumorigenicity (82% or 89% accuracy) using Affymetrix transcript profiling of the livers of rats exposed to chemicals at one dose for 4 days
- Transcript profiles of Affymetrix and RNA-seq have comparable results in biomarker TAL ranges of -Log(p-values) indicating that the TALs can be used for prediction using RNA-seq data (data not shown)
- Using TempO-Seq transcript profiles, the NAM can predict tumorigenicity of chemicals and their dose levels (80-91% accuracy)

The NAM can identify

- Doses that would induce tumors in the livers of rats
- The mode of action of the chemical
- By inference from the identified mode of action, the human relevance of the predictions based on current understanding

This information (along with histopathology, genetic toxicity, and hormonal perturbation) could be used to determine whether a 2-year bioassay is warranted or if the information supports a waiver from requiring a 2-year bioassay.

Acknowledgments

We would like to thank those involved in the rat 4-day studies (Glenda Moser, Nicholas J. Machesky, Jennifer A. Price, Morgan Q.S. Wenling, Carol L. Sabourin, Milton R. Hejtmancik, Molly Vallant), and Molly Windsor for assistance in making the figures.

Disclaimer

The information in this document has been funded in part by the U.S. Environmental Protection Agency. It has been subjected to review by the Center for Computational Toxicology and Exposure and approved for publication. Approval does not signify that the contents reflect the views of the Agency, nor does mention of trade names or commercial products constitute endorsement or recommendation for use.