IDENTIFICATION OF TRANSCRIPTIONAL NETWORKS INVOLVED IN PEROXISOME PROLIFERATOR CHEMICAL-INDUCED HEPATOCYTE PROLIFERATION.

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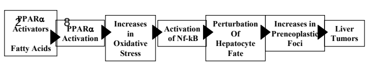
ABSTRACT

Peroxisome proliferator chemical (PPC) exposure leads to increases in rodent liver tumors through a non-genotoxic mode of action (MOA). The PPC MOA includes increased oxidative stress, hepatocyte proliferation and decreased apoptosis. We investigated the putative genetic regulatory events leading to hepatocellular proliferation following short-term exposure to the PPC di-2-ethylhexyl phthalate (DEHP). Male B6C3F1 mice were exposed to DEHP via oral gavages at a single concentration of 1150 mg/kg/day and sacrificed after 2, 8, 24 and 72 hours. Liver samples were analyzed using the Affymetrix Mouse 430_2 genechip. We identified 794 (2hr), 1921 (8hr), 2019 (24hr) and 2076 (72hr) transcript clusters to be statistically significant. Cell proliferation was markedly increased at 72 hrs. Analyzing differentially expressed genes (DEGs) in the context of cell proliferation showed roughly 10% to be related to cell cycle at each time point with very little overlap across the time points, suggesting sequential non-overlapping regulatory events controlling cell cycle gene expression. Putative transcription factor binding sites were predicted in the 10 Kb 5' upstream region of DEG's at each time point by sequence analysis (using the TRANSFAC® 2008.2 database). Statistically significant transcriptional sub networks were inferred computationally from putative genetic-regulatory interactions. The putative transcription networks of regulated cell cycle genes were compared with published data. This work identified a number of transcription factors that may underlie the increases in cell proliferation observed upon PPC exposure.

BACKGROUND

Di-(2-ethylhexyl)phthalate (DEHP) is an environmentally-relevant chemical (plasticizer) as well as a PPARα activator. After chronic exposure in mice and rat increases the incidence of liver tumours. The mode of action of DEHP likely involves increases in oxidative stress, increases in cell proliferation and decreased apoptosis. Cell proliferation is determined by changes in genes which regulate the cell cycle. Our current goal is to derive generic networks leading to increased cell proliferation across time points using gene expression data that can describe PPC mode of action.



STUDY DESIGN

Wild type male B6C3F1 mice were treated with 1150mg/kg of DEHP or Corn oil carrier each day. Treated and control animals were sacrificed 2hr, 8hr, 24hr and 72hr after dosing. Microarray analysis of the tissue samples were analyzed using Affy MOE430_2 chips (3/group)

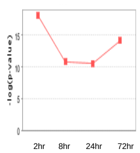
ANALYSIS OF GENE EXPRESSION

Data output files (.cel) from microarray studies which utilized DNA chips from Affymetrix were first analyzed by Bioconductor package Simpleaffy to assess data quality. Data (.cel files) was analyzed and statistically filtered using Rosetta Resolver® version 7.1 software (Rosetta Biosoftware, Seattle, WA). Statistically significant genes were identified using one-way ANOVA with a false discovery rate (Benjamini-Hochberg test) of ≤ 0.05 followed by a post-hoc test (Scheffe) for significance. Genes identified were significantly perturbed at least one time point in 2h, 8h, 24h, or 72h and also identified from it a subset for proteins regulating/in cell cycle. Significant transcripts were evaluated for relevance to canonical pathways and biological functions using Ingenuity Pathways Analysis (Ingenuity Systems, www.ingenuity.com). Significant gene lists from ANOVA analyses were compared at the Gene Ontology level using MetaCore from GeneGo, Inc (http://trials.genego.com/cgi/index.cgi#Information).

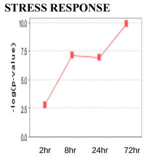
Identification of over-represented putative transcription factor binding sites in the network were identified using oPOSSUM. This is an integrated system that searches for evidence of co-regulation by one or more transcription factors (TFs). oPOSSUM combines a pre-computed database of conserved TFBSs in human and mouse promoters with statistical methods for identification of sites over-represented in a set of co-expressed genes. (http://www.cisreg.ca/oPOSSUM/) Literature based networks were built from cell cycle and related genes across all time points using Agilent Literature Search Software in conjunction with Cytoscape v2.2.

FUNCTIONAL ANALYSIS OF DIFFERENTIALLY EXPRESSED GENES

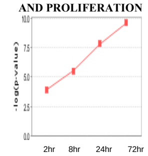
LIPID METABOLISM



NRF2-MEDIATED OXIDATIVE STRESS RESPONSE



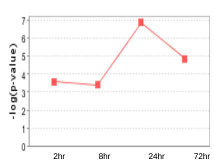
CELLULAR GROWTH AND PROLIFERATION

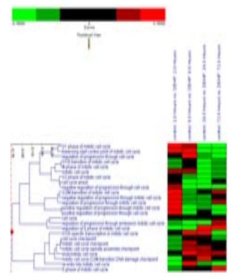


Lipid metabolism, Oxidative stress response and cellular growth & proliferation to be significant across all time points

FUNCTIONAL AND GO LEVEL ANALYSIS OF CELL CYCLE GENES ACROSS TIME POINTS

CELL CYCLE





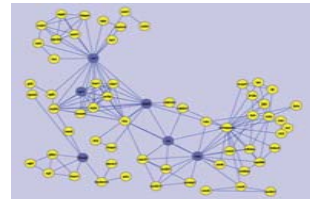
Cell cycle related gene expression changes to be significant across all time points

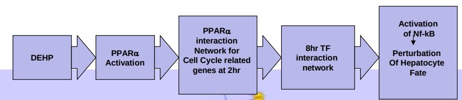
PREDICTED GENETIC REGULATORY INTERACTIONS FOR CELL CYCLE DEG's AND PPARα INTERACTIONS FOR EACH TIME POINT

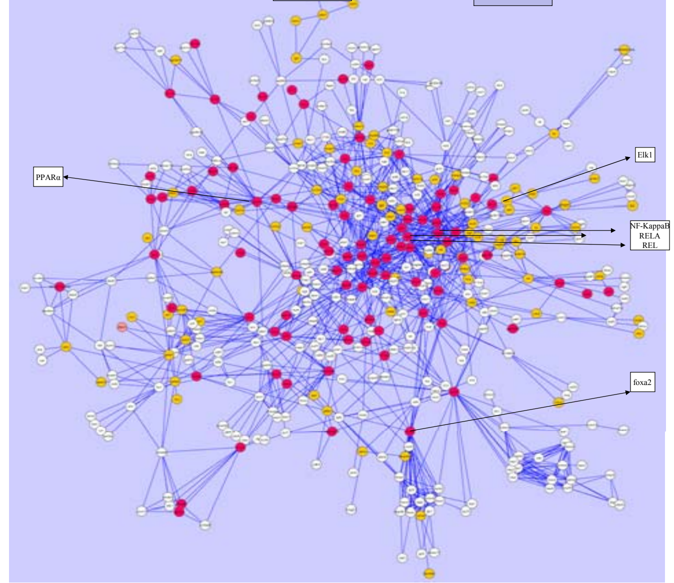
Predicted networks were generated at each time point for Cell cycle genes and their PPAR-alpha interaction. Over-represented TF's were identified for each predicted network

2hr-network	8hr-network	24hr-network	72hr-network
RELA	RELA	RELA	RELA
REL	REL	REL	REL
	MZF1_1-4	MZF1_1-4	MZF1_1-4
	SP1	SP1	SP1
	dl_1	dl_1	dl_1
	MZF1_5-13	MZF1_5-13	MZF1_5-13
	Eip74EF	Eip74EF	Eip74EF
	ELK1	ELK1	ELK1
	Dl_2	Dl_2	
	ELF5	ELF5	
	ZNF354C	ZNF354C	
	NF-kappaB	NF-kappaB	
	usp		usp
	SPIB		
	IRF1		
	Dl		
	NFKB1		
	HNF4A		
	TLX1-NFIC		
		IRF2	IRF2
			AB4
SRF			
Foxa2			

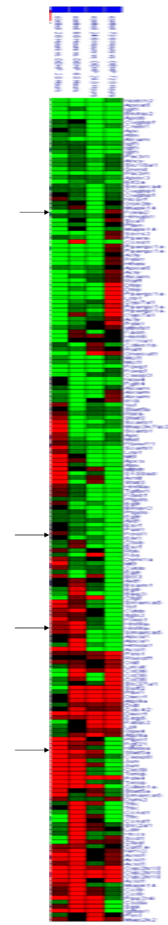
Foxa2, SRF and RELA may be important early regulators of PPAR-alpha mediated cell cycle activation. Literature based network evidence for SRF, Rela, and NF-Kappab interaction,







Red – Genes in the 'PPARα interaction Network for Cell Cycle related genes at 2hr '
Yellow- Genes in the' 8hr TF interaction network'



Associated processes for all genes in the cluster

cell cycle	30
cell proliferation	30
lipid metabolic process	35
response to stress	36
apoptosis	38
programmed cell death	38
cell death	40
cell development	55
cell differentiation	74
cell communication	80

CONCLUSION

Putative genetic networks can be linked to gene expression changes to describe cell cycle related processes that is in agreement with PPC mode of action Lipid metabolism-> oxidative stress->cell cycle/apoptosis/cell proliferation.