SEURAT-1

Towards the Replacement of in vivo Repeated Dose Systemic Toxicity Testing

DETECTIVE

Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

Molecular mechanisms of chemical injury: Input from epigenomics

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UM

TOXICOGENOMICS

MAASTRICHTUNIVERSITY

The epigenome

- The epigenome consists of a record of the chemical changes to the DNA and histone proteins of an organism which can be passed down to an organism's offspring.
- Changes to the epigenome can result in changes to the structure of chromatin and changes to the function of the genome.
- Unlike the underlying genome which is largely static within an individual*, the epigenome can be dynamically altered by environmental conditions.*

Chemical Research in Toxicology

RAPID REPORT

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Epigenetic Changes in Individuals with Arsenicosis

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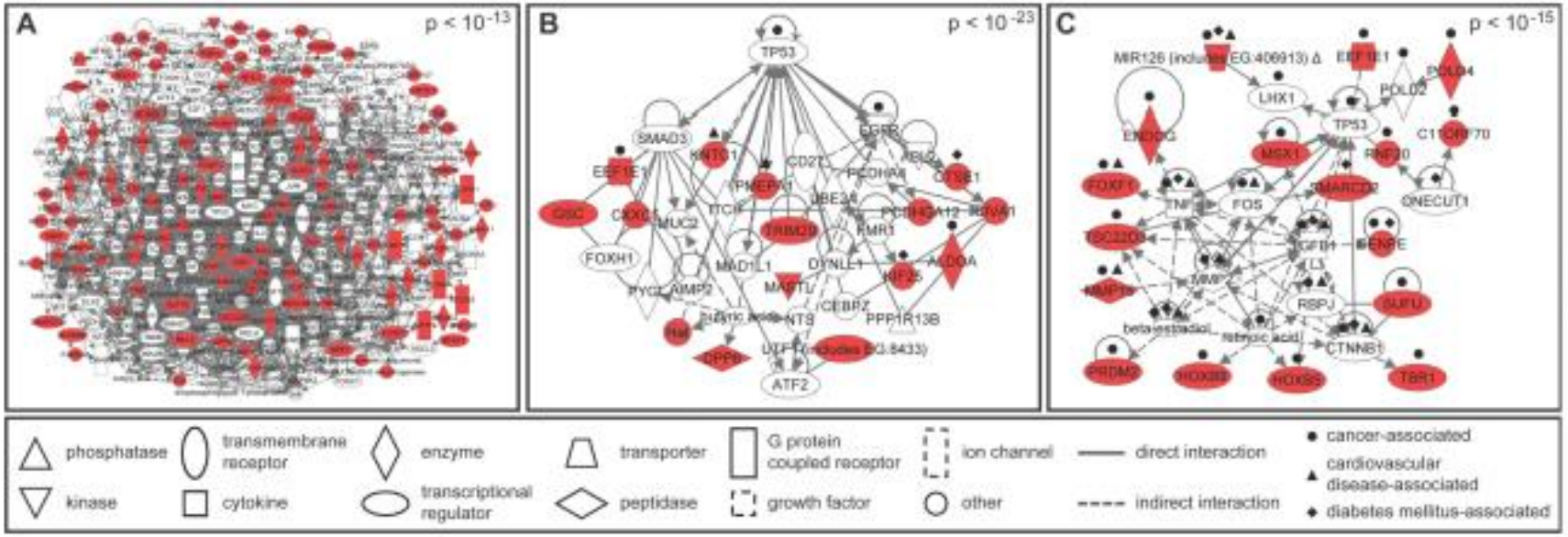

Figure 2. Epigenetically modified iAs-induced networks. (A) Large interacting network of hypermethylated genes. (B) Tumor protein p53 (tp53)-associated network. (C) The iAs-induced tumor suppressorome. *P*-values are shown in the top right corners of each network. Networks are displayed with symbols representing products of hypermethylated genes (red symbols) or the proteins associated with these genes (clear symbols).

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INORGANIC COMPOUNDS

Epigenetic mechanisms underlying arsenic-associated lung carcinogenesis

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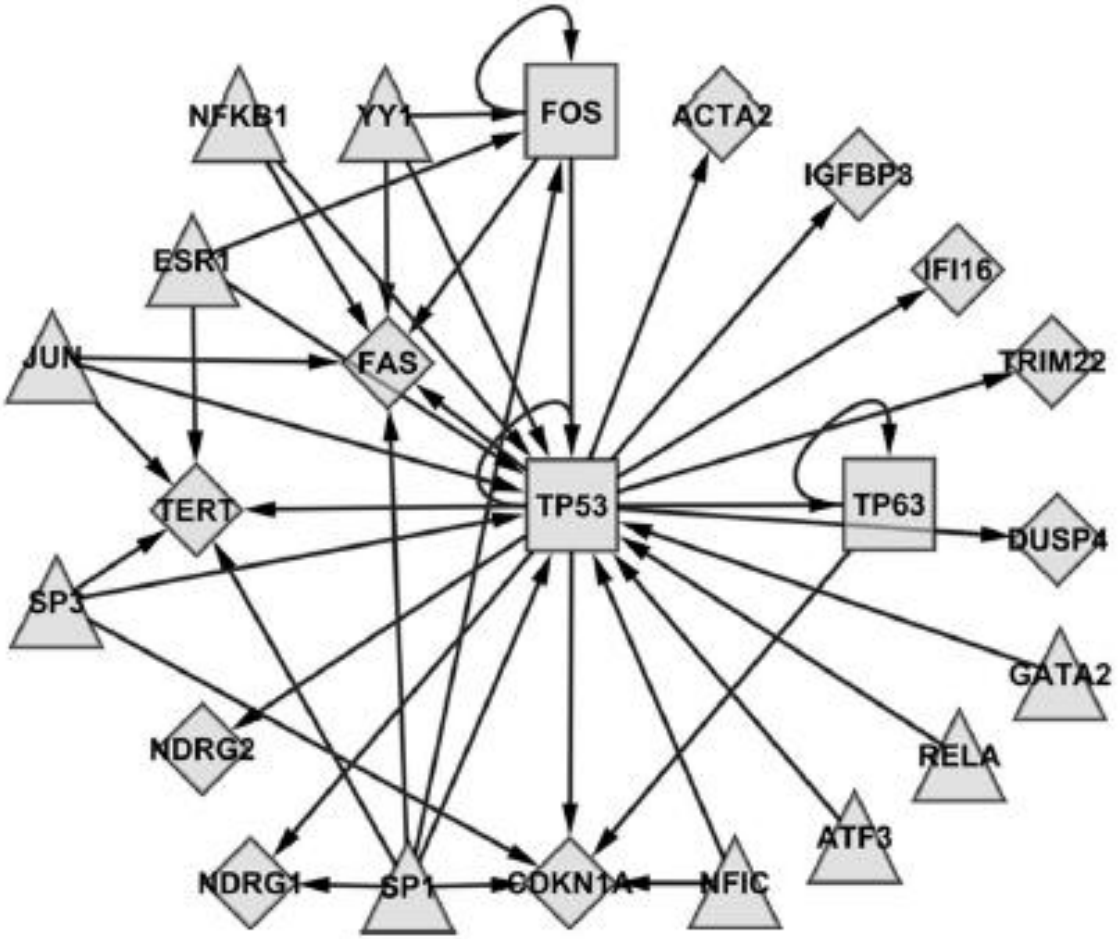
- A549 human adenocarcinoma lung cells were exposed to a low (0.08 µM), intermediate (0.4 µM) and high (2 µM) concentration of sodium arsenite for 1, 2 and 8 weeks.
- DNA was isolated for whole-genome DNA methylation analyses using NimbleGen 2.1 M deluxe promoter arrays, and MeDIP=chip analysis was performed
- RNA was isolated for whole genome transcriptomic analysis using Affymetrix microarrays.

Results -1

- A dose–response methylation effect for 851 genes at all three time points.
- A time dependent dose–response in methylation for 94 genes.
- For 24 genes, inverse relation between changes in DNA methylation and gene expression
- As modulated genes which function as transcription factor, thereby affecting target genes which are known to play a role in lung cancer promotion and progression
- A tumor protein p53 (TP53) subnetwork was identified, showing the interactions of TP53 with other genes affected by As

Results -2



TP53 subnetwork showing the interactions of *TP53* with other genes induced by As affecting target genes which play a role in lung cancer promotion and progression

Research questions

DETECTIVE
Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

- Can the epigenome be induced by repeated dosing of hepatotoxicants *in vitro* in primary human hepatocytes ?
- Are such changes persistent after terminating toxic treatment?

  - Prototypical liver toxicants AFB1, CsA and VPA
  - PHHs pooled from 3 donors, cultured in collagen sandwich configuration
  - Daily toxicant administration for 5 days, followed by a 3 days washout
  - Cross-omics analysis (methyl DNA – mRNA – microRNAs)
  - *In vitro/in vivo* translation by using gene expression profiles from patients livers

Assessment of repeated dose toxicity of AflatoxinB1 in pooled human primary hepatocytes using integrative 'omics data analyses

- Hepatotoxic and carcinogenic mycotoxin
- Acute: apoptosis of liver cells and bile duct proliferation (Aflatoxicosis)
- Chronic: hepatocellular carcinoma
- AFB1 exposure is associated with global hypo-methylation and gene-specific hypermethylation

Results: numbers of modulated genes

Number of DMGs, DEGs, and DE-miRNAs in PHH after 5 days of exposure to the high dose (1 µM) and low dose (0.3125 µM) of AFB1, and after a washout of 3 days

	High dose					Low dose					
Direction of effect*	DMG	DEG		DE-miRs		DMG		DEG		DE-miRs	
	Magnitude >0 or <0; p-value <0.01 FDR <0.05	P-value <0.05; FDR <0.05; FC >1.5 or <-1.5		P-value <0.05; FDR <0.05; FC >1.5 or <-1.5		Magnitude >0 or <0; p-value <0.01 FDR <0.05		P-value <0.05; FDR <0.05; FC >1.5 or <-1.5		P-value <0.05; FDR <0.05; FC >1.5 or <-1.5	
	5 days	5 days	washout	5 days	washout	5 days	washout	5 days	washout	5 days	washout
+	2511	1399	896	15	8	1896	4397	702	368	0	0
-	2491	1156	1069	4	9	3743	3734	788	528	0	0
Total	5002	2555	1965	19	17	5639	8131	1490	896	0	0

*direction of effect:

+ = DNA hypermethylation; gene expression upregulation; miRNA expression upregulation

- = DNA hypomethylation; gene expression downregulation; miRNA expression downregulation

A) 2 persistently hypermethylated - downregulated genes and B) 16 persistently hypomethylated – upregulated genes, following 5 days of exposure to 0.3 µM of AFB1 and 3 days of wash-out

A.

Entrez Gene ID	Gene name	FC 5D	p-val 5D	FC WO	p-val WO
10974	ADIRF	-1.7305	0.0021	-2.1523	0.0000
6768	ST14	-1.5584	0.0055	-1.5868	0.0002

B.

Entrez Gene ID	Gene name	FC 5D	p-val 5D	FC WO	p-val WO
5111	PCNA	2.1010	0.0044	1.7179	0.0000
7296	TXNRD1	2.2622	0.0031	1.6844	0.0000
8812	CCNK	1.6374	0.0021	1.5481	0.0000
81624	DIAPH3	1.6981	0.0125	1.5173	0.0002
5874	RAB27B	2.7440	0.0029	2.7740	0.0000
8343	HIST1H2BF	2.5235	0.0025	1.8298	0.0000
8351	HIST1H3D	3.5308	0.0018	2.7170	0.0000
5678	PSG9	1.9268	0.0125	1.8668	0.0030
84675	TRIM55	1.6709	0.0045	1.8220	0.0000
51232	CRIM1	1.7854	0.0066	1.7126	0.0001
169792	GLIS3	1.7870	0.0035	1.6761	0.0000
90627	STARD13	1.7390	0.0031	1.6230	0.0000
196	AHR	1.8178	0.0398	1.5874	0.0032
11167	FSTL1	1.7953	0.0019	1.5646	0.0001
3486	IGFBP3	1.7002	0.0027	1.5607	0.0000
9173	IL1RL1	2.1095	0.0064	1.5003	0.0002

- DNA damage response
- Cell growth
- Metastatic events

Assessment of repeated dose toxicity of Cyclosporine A in pooled human primary hepatocytes using integrative 'omics data analyses

- neutral highly lipophilic cyclic endecapeptide and a potent immunosuppressant
- CsA is a multidrug resistance-1 protein (MDR1) substrate
- contributed significantly to progress in organ transplantation but also induces various toxic effects in the kidney and the liver
- cholestasis is a well known problem after organ transplantation
- inhibits the bile-acid secretion and thus can cause cholestatic liver injury

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Results: numbers of genes modulated in PHH by 30 µM of CsA

	3-day compound exposure			5-day compound exposure			3-days washout period after 5-day compound exposure		
	DMG	DEGs	DE-miRs	DMG	DEGs	DE-miRs	DMG	DEGs	DE-miRs
Direction of effect *	▪ Magnitude >0 or <0; ▪ P-value <0.01 ▪ FDR <0.05	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5	▪ Magnitude >0 or <0; ▪ P-value <0.01 ▪ FDR <0.05	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5	▪ Magnitude >0 or <0; ▪ P-value <0.01 ▪ FDR <0.05	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5	▪ P-value <0.05; ▪ FDR <0.05; ▪ |FC| >1.5
+	222	576	10	11	741	12	46	787	9
-	254	777	12	9	736	17	86	847	9
Total	**476**	**1353**	**22**	**20**	**1487**	**29**	**132**	**1634**	**18**

Cell cycle

6 persistently changed
↓
22 persistent DEGs

SEVENTH FRAMEWORK PROGRAMME

Cosmetics Europe
the personal care association

Assessment of repeated dose toxicity of valproic acid in pooled human primary hepatocytes using integrative 'omics data analyses

- VPA is known to induce liver steatosis, presumably through oxidative stress
- inhibits the enzyme histone deacetylase 1, thereby inducing histone hyperacetylation
- stimulates active demethylation in a replication independent manner by increasing accessibility of demethylase enzyme
- effects on mRNA and miRNA expression

DETECTIVE
Detection of Endpoints and Biomarkers for Repeated Dose Toxicity Using In Vitro Systems

Number of DMGs, DEGs, and DE-miRs in PHH after 5 days of exposure to 15 mM VPA and after a 3-days washout period

	5-day compound exposure			3-days washout period after 5-day compound exposure		
	DMG	DEG	DE-miRs	DMG	DEG	DE-miRs
Direction of effect*	Magnitude >0 or <0; p-value <0.01 FDR <0.05	P-value <0.05; FDR <0.05; |FC| >1.5	P-value <0.05; FDR <0.05; |FC| >1.5	Magnitude >0 or <0; p-value <0.01 FDR <0.05	P-value <0.05; FDR <0.05; |FC| > 1.5	P-value <0.05; FDR <0.05; |FC| >1.5
+	5251	1932	30	1798	93	38
-	3373	1478	29	2022	163	12
Total	8624	3410	59	3820	256	50

577 persistent DMGs
152 persistent DEGs
18 persistent DE-miRs

SEVENTH FRAMEWORK PROGRAMME

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Conclusions

- In a repeated dose regime the epigenome of PHHs appears inducible by liver toxicants, which to some extent is concordant with induced gene expression changes.
- Epigenomic responses to prototypical toxic challenges appear highly dynamic, but also to some extent persistent.
- Novel response patterns have been disclosed which to some extent can be translated to gene signatures from diseased livers.
- Promising biomarkers for repeated dose toxicity in humans have been identified
- Follow-up is required which in particular should consider
  - Larger numbers of chemicals for training and validating the predictive models
  - Physiologically relevant doses
  - Cross-omics time series analysis for inferring causality

Many thanks to:

- The people of the Dept of Toxicogenomics at Maastricht University
- The DETECTIVE consortium
- The European Commission