Towards distinguishing adaptive and adverse response to chemicals using gene expression data

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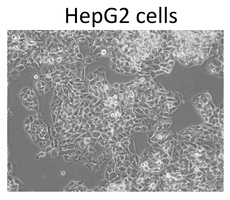
ABSTRACT

Gene expression data is increasingly used for risk assessment due to both its ease of generation as well as its ability to provide mechanistic insight into compound action. Current methods rely on modelling the dose-response characteristics present in gene expression data to identify points of departure in order to define the lowest dose of a compound that might trigger a transcriptional response (the No Observed Transcriptional Effect Level/NOTEL). However, this approach does not discriminate appropriately between adaptive and toxic responses as it includes *all* transcriptional activity. In risk assessment as well as in drug development we may end up stopping the testing of a given compound/drug because of its biological activity even if that activity is not of concern. In this context, developing a framework with the ability to discriminate between compound adaptive and adverse effects is of paramount importance for improving current risk assessment strategies for chemicals and drugs.

MATERIALS & METHODS

HepG2 cells



24h exposure

Compound	Dose (uM)
4-hexylresorcinol	0.1, 0.4, 1, 4, 10, 40
4-propylphenol	0.4, 1, 4, 10, 40, 100
4-butylphenol	0.4, 1, 4, 10, 40, 100
4-nonylphenol	0.1, 0.4, 1, 4, 10, 20
p-cresol	0.4, 1, 4, 10, 40, 100
control	0

BioSpyder
Targeted sequencing
~3,000 genes

Raw data processing and QC

Identifying and functionally annotating modules of highly correlated genes by Weighted gene co-expression network analysis (WGCNA)[1]

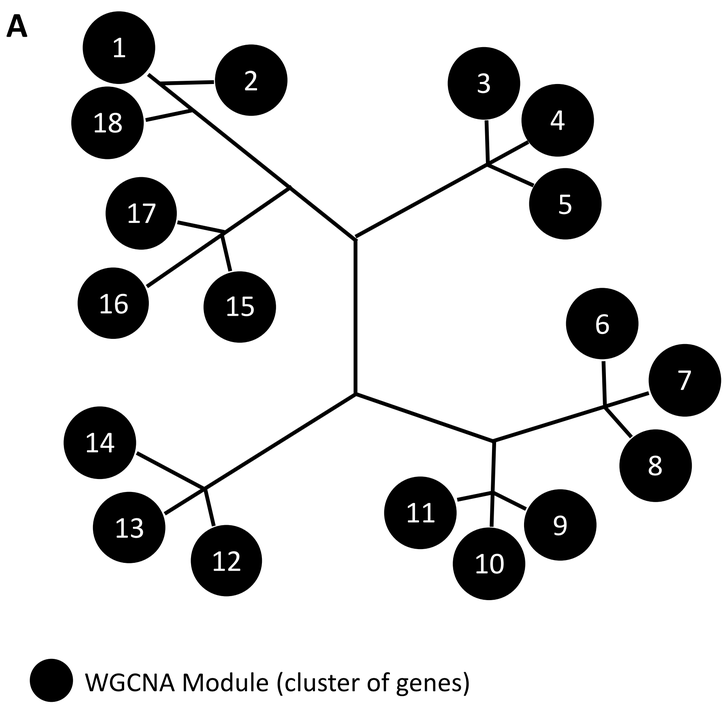
Dose-specific modulation of the different modules

Identifying differentially expressed genes (DEGs)

WGCNA modules enrichment of DEGs by gene set enrichment analysis (GSEA)[2]

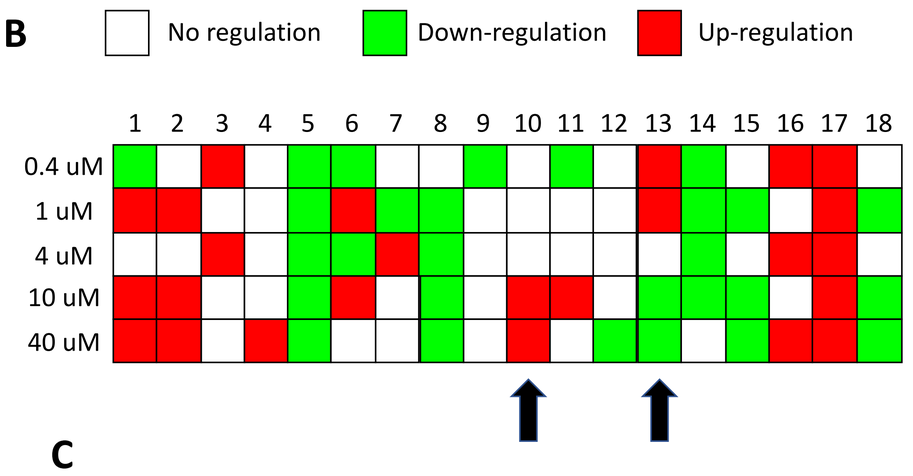
RESULTS

A



WGCNA Module (cluster of genes)

B



C

Module	Function (GO:BP)	Pvalue
13	Cellular response to stress	8.8×10^{-4}
	Cellular response to hypoxia	8.8×10^{-4}
	Cellular response to decreased oxygen levels	1.1×10^{-3}
	Cellular response to oxygen levels	1.8×10^{-3}
10	Apoptotic process	1.6×10^{-2}
	Cell death	1.8×10^{-2}

- Taking the p-cresol as case study and by mapping the compound dose-specific responses onto the network obtained by the WGCNA (**A**), we identified modules of interest whose modulation is influenced by the dose (**B**)
- Modules are used to categorise compounds activity as adaptive or adverse
- Module 13 was found to be up-regulated at low doses switching to down-regulation when increasing the dose of exposure (**B**). This module was found to be associated with biological activity linked to cell stress (general stress and hypoxic conditions) (**C**)
- Module 10 was found to undergo up-regulation at high doses of exposure (**B**) and its biological activity was found to be associated with cell death (mainly apoptosis) (**C**)

DISCUSSION

- Current challenges in discriminating adaptive and adverse responses include a lack of comprehensive understanding about stress pathways and their drivers
- Correlation-based approaches represent a valuable solution as they allow the analysis of multiple conditions at the same time (compounds, doses and timepoints) and are not biased by existing knowledge
- Our approach have proven valuable in identifying transcriptional modules activated at the different exposure doses
- Combining this approach with an improved biological understanding of stress pathways may pose the basis towards the development of a framework able to identify tipping points where there is a switch from adaptive to adverse responses with the long term goal of refining of current risk assessment strategies for chemicals and drugs

REFERENCES

[1]WGCNA: An R Package for Weighted Correlation Network Analysis. Langfelder P. and Horvath S. (2008). *BMC Bioinformatics*. 9:559.
[2]Gene set enrichment analysis: A knowledge-based approach for interpreting genome-wide expression profiles. Subramanian A. et al. (2005). *PNAS.* 102 (43) 15545-15550.