GENOMIC APPROACHES FOR RELATIVE POTENCY ASSESSMENT

Thomas, R.S.[1], Rowlands, J.C.[2], Budinsky, R.A.[2], Thompson, C.M.[3], Urban, J.D.[3], and Dombkowski, A.[4]
[1]The Hamner Institutes For Health Sciences, Research Triangle Park, NC 27709; [2]The Dow Chemical Company, Midland, MI 48674; [3]ToxStrategies, Austin, TX 78731; [4]Wayne State University, Detroit, MI 48201

Introduction: Toxicogenomics holds great promise for improving our understanding of the mode or modes of action (MOA) for environmental toxicants [1]. Advances in microarray technology now include the capability to survey the entire transcriptome providing a global evaluation of cellular changes. The technology has also become reliable and reproducible. Small changes in gene expression can be reliably detected and extensive validation studies performed by the MicroArray Quality Control (MAQC) project demonstrated consistent results across multiple platforms and research sites [2]. Observed molecular changes include both the direct and indirect effects of the chemical on the cell or tissue. The direct effects include potential key events in the mode of action (MOA) for a chemical while the indirect effects include secondary processes that are activated following the initial key events. In either case, toxicity would not occur without alterations in the transcriptional program [3].

When genomics assays are conducted using a dose-response approach, gene expression microarray analysis can provide both quantitative and qualitative information on the dose at which cellular processes are affected and allow a global assessment of relative potencies that cannot be captured using a single gene or apical response. As such, genomic technology can provide a sensitive and comprehensive examination of the molecular changes resulting from exposure to dioxin-like compounds, and specifically can be used to evaluate differences in relative potency. In this study, effects on the transcriptome associated with exposures to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), 2,3,4,7,8-pentachlorodibenzofuran (4-PeCDF), or 2,3,7,8-tetrachlorodibenzofuran (TCDF) in primary cultures of human and rat hepatocytes were evaluated using microarray analyses. Relative potencies were then evaluated via Benchmark Dose (BMD) analyses.

Materials and Methods: Primary cultures of human and rat hepatocytes were exposed to 7 log doses from 0.0001 to 100 nM of TCDD, 4-PeCDF, or TCDF for 24 hours. A vehicle control (DMSO) was also included. Following exposure, cell viability was assessed based on lactate dehydrogenase (LDH) release and adenosine triphosphase (ATP) content. RNA was isolated from the cells and used to examine genome-wide expression changes on Agilent rat and human whole genome 4x44K oligonucleotide microarrays. The gene expression data was normalized based on the median signal values and $\log_2$ transformed. The probes were then filtered to remove those with signal values at or below background. Microarrays were scanned using an Agilent® dual laser DNA microarray scanner, model G2565AA, with 5 mm resolution. Microarray image analysis was performed with Agilent® Feature Extraction software, version 9.5.1. Intensity data were imported into Rosetta Resolver (version 5.1) for analysis. Transcripts were determined to be "present" by using a statistical test based on spot (feature) intensity, pixel variance, and background intensity. Transcripts with a p-value ≤ 0.001 were specified as present. For each comparison between treated samples and DMSO controls, transcripts absent in both treated and DMSO controls were excluded from subsequent analysis. One-way analysis of variance (ANOVA) was performed between each treatment and DMSO controls to identify transcripts with a statistically significant difference in expression (altered $\geq |1.5$ fold change$|$, $p < 0.05$.

BMD analyses of the gene expression microarray data was performed as previously described [4-5] with modification. The $\log_2$ transformed expression values for each probe were fit to a series of five different dose-response models - Hill, power, linear (1°), polynomial 2°, and polynomial 3°. Each model was run assuming constant variance and the benchmark response (BMR) factor was set to 1.349 multiplied by the standard deviation in control animals to estimate a BMD with a 10% increase in tail area [4-5]. For model selection, a nested likelihood ratio test was performed on the linear, polynomial 2°, and polynomial 3° models. If the more complex model

provided a significantly improved fit ($p < 0.05$), the more complex model was selected. The simpler model was selected if the more complex model did not provide a significantly improved fit ($p \geq 0.05$). The Akaike information criterion (AIC) for the selected polynomial model was then compared with the AIC for the Hill and power models. The model with the lowest AIC was selected as the final model except in cases where the 'k' parameter for the Hill model was less than one-third the lowest treatment concentration (0.00001 nM). In the cases where the 'k' parameter for the Hill model was out of bounds, the Hill model was excluded from the final selection. The final selected model was used to calculate a BMD and BMDL. To avoid model extrapolation and any potential bias from poor fitting genes, probes with a BMD value greater than the highest treatment concentration (100 nM) or a goodness-of-fit p-value < 0.1 were removed from further analysis. The remaining probes were converted into unique genes based on their NCBI Entrez Gene ID. When two or more probe sets were associated with a single gene, the BMD and BMDL values for the individual probes were averaged to obtain a single BMD and BMDL value. The Entrez Gene identifiers were then matched to their corresponding canonical signaling pathways using the GeneGo Metacore database (GeneGo, St. Joseph, MI). The median BMD and BMDL values were used to summarize each signaling pathway. A comparison of the relative potencies across the AHR ligands and across species was performed for the different genes and signaling pathways.

Results and Discussion:

The preliminary results demonstrate that TCDD, TCDF, and 4-PeCDF significantly affected the transcription of a limited number of genes, and most of the gene alterations were congener-specific. These observations suggest that slight structural differences between DLCs might allow for differential and selective modulation of gene expression and thus different apical effects. Among the annotated genes differentially expressed by DLCs, a relatively small percentage of the orthologs were differentially expressed in both rat and human hepatocytes. These findings suggest species differences in response to DLCs. Benchmark dose (BMD) analysis of gene expression changes showed that there were several-fold differences in potency across species with respect to many individual orthologs as well as GeneGo Metacore canonical signaling pathways. Overall, the rat hepatocytes were observed to be more sensitive than the human hepatocytes. Taken together, there were species differences in both sensitivity and overall response to DLCs. Additional analyses will further explore the derivation of relative potency estimates by using the BMD values for canonical signaling pathways. Such genomics-based REP values may inform future evaluations of TEF values, and have important implications for human health risk assessment.

Acknowledgements:

This research was funded by The Dow Chemical Company.

References:

1. Nuwaysir, E.F., et al., *Microarrays and toxicology: the advent of toxicogenomics.* Mol Carcinog, 1999. **24**(3): p. 153-9.
2. Shi, L., et al., *The MicroArray Quality Control (MAQC) project shows inter- and intraplatform reproducibility of gene expression measurements.* Nat Biotechnol, 2006. **24**(9): p. 1151-61.
3. Farr, S. and R.T. Dunn, 2nd, *Concise review: gene expression applied to toxicology.* Toxicol Sci, 1999. **50**(1): p. 1-9.
4. Yang, L., et al., *BMDExpress: a software tool for the benchmark dose analyses of genomic data*. BMC Genomics, 2007. 8(387): p 1-8.
5. Thomas, R.S., et al., A Method to Integrate Benchmark Dose Estimates with Genomic Data to Assess the Functional Effects of Chemical Exposure. Toxicol Sci, 2007. **98**(1): p. 240-248.