2,3,7,8-TETRACHLORODIBENZO-P-DIOXIN ALTERS THE EXPRESSION OF FUNCTION-REGULATORY GENE CLUSTERS IN HUMAN MICROVASCULAR ENDOTHELIAL CELLS.

Diane E. Heck [1], Laura Davison [1], Peihong Zhou [1], Marion Gordon [1] and Jeffrey D. Laskin [2]
[1]Departments of Pharmacology and Toxicology, Rutgers University and [2] Environmental and Community Medicine, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ USA 08854.

Introduction

Exposure of humans and other mammals to the environmental contaminant and putative human carcinogen 2,3,7,8-Tetrachlorodibenzo-p-dioxin (TCDD) has been associated with cardiovascular disease, edema, hypertriglyceridemia and congenital hemangiomas.[1-4] In addition, exposure to TCDD has been found to mediate endothelial cell proliferation, increases in microvascular permeability, and deposition of a pro-angiogenic extracellular fibrin matrix, processes which are also critical to tumor progression.[5-8] Mice lacking the TCDD-liganded aryl hydrocarbon accessory protein ARNT exhibit defective angiogenesis, stunted development and embryo wasting.[9] These findings point to the vascular endothelium as a potential target important in TCDD mediated toxicity. To examine the effects of TCDD on the human endothelium, primary cultures of human microvascular endothelial cells were treated with TCDD and analyzed for genome-wide changes in gene expression using nucleic acid based microarrays.

Results and Discussion

Of the 8,514 unique genes/gene clusters analyzed following treatment of human microvascular endothelial cells with TCDD (10 nM, 5h), 582 specific mRNA sequences were significantly altered. However, a method of analysis that would be likely to provide information on the metabolic processes affected by TCDD was required to understand the significance of these alterations. Although multiple data sets derived from microarray experiments have been analyzed using established techniques in unsupervised multivariate analysis, at the current time there is no established standard method for analyzing singular data sets.[10-12] To address these issues we devised a systematic qualitative approach. In our initial experiments, we uncovered a cluster of TCDD-regulated genes important in tissue specific functions of the vasculature using this supervised knowledge-based approach. This analysis system evaluates alterations in gene expression in a binary manner, that is in comparing mRNA levels from two groups (in this case untreated and TCDD-treated), a score of 1 is given for any significant alteration and a score of 0 for no significant alterations. Beyond the technically driven decision to determine the fold increase that will be considered significant, the relative levels or frequency of the message changes are not considered, therefore we refer to the method as ungraded. The scores were then associated with specific gene groups identified using the public databases. In this manner the percentage of genes in a specific group exhibiting altered mRNA expression was determined. In our analysis, we then ranked the percentage of genes/group exhibiting mRNA alterations in order of magnitude. Results from our analysis indicated that of the 586 genes we identified as altered in expression following TCDD treatment, the largest group, 27.1% of the affected mRNAs, were either ESTs similar to proteins included in this group, or encoded various characterized structural and localized proteins. We designated the set of characterized genes contained in this group as Endothelial Cluster

1(EC1). Of the proteins included in this group, 53.8% were important in nutrient and solute transport, 18.8% function in cytoskeletal roles, and14.7% mediate lipid signaling and metabolism.

Results of this analysis indicates that exposure to TCDD alters mRNA levels for EC1, a unique cluster of 112 genes important in solute and nutrient transport, lipid metabolism and vascular structure. Many of these genes were not previously recognized as modulated by TCDD and a role for TCDD in regulating these processes has not been reported. In further experiments, we confirmed that TCDD induced changes in mRNA expression levels of representative genes from EC1 using RT-PCR. In these experiments expression of mRNAs for NOS2, NOS3, VEGF, and PLA2 2B were examined. mRNA levels were analyzed at various times (0-72 h) following TCDD treatment. Levels of the mRNAs 5 hours after TCDD treatment were found to be similar to those derived from the microarray analysis.

Our results indicate that TCDD plays a significant role in regulation of the expression of genes that mediate the fundamental processes of nutrient and solute transport, lipid metabolism and cell shape and structure in human endothelial cells. We hypothesize that TCDD induced changes in the mRNA levels of the 112 genes which comprise this cluster reflect cellular responses to perturbations in metabolic processes mediated by the proteins encoded by these mRNAs; and that altered expression of these proteins mediate changes in microvascular function which, in turn, results in TCDD-induced vascular pathology.

Methods and Materials

mRNA was isolated using guanidine thiocyanate and oligo dT cellulose (Quiagen) and microarray analysis performed by Incyte Pharmaceuticals.[13] alterations in gene expression were confirmed by semi-quantitative RT-PCR as previously described.[14]

References

1. Guiney P.D., Smolowitz R.M., Peterson R.E. and Stegeman J.J. (1997) Tox. Applied Pharm. 143, 256-273.
2. 2. Gannon M., Gilday D. and Rifkind, A.B. (2000) Tox. Applied Pharm. 164, 24-37.
3. Brewster D.W., Bombick D.W. and Matsumura F. (1988) J. Toxicol Environ Health. 25, 495-507.
4. Mocarelli P., Marocchi A., Brambilla P., Gerthoux P.M., Colombo L., Mondonico A. and Meazza L. in Gallo M.A., Scheuplein R.J, and Van Der Heijden Biological Basis for Risk Assessment of Dioxins and Related Compounds (1991) Cold Spring Harbor Laboratory Press. 95-107.
5. Bruner-Tran K.L., Rier S.E., Eisenberg E. and Osteen K.G. (1999) Gynecol Obstet Invest. S1, 45-56.
6. Abbott B.D., Morgan K.S., Birnbaum L.S. and Pratt R.M. (1987) Teratology. 35, 335-344.
7. Bagchi D., Bagchi M., Tang L. and Stohs S.J. (1997) Toxicol Lett. 14, 31-7.
8. Dvorak H.F. (2000) Semin Perinatol. 24, 75-8.
9. Maltepe E., Schmidt J.V., Baunoch D., Bradfield C.A. and Simon M.C. (1997) Nature. 386, 403-7.
10. Eisen MB, Spellman PT, Brown PO and Botstein D. (1998) Proc Natl Acad Sci U S A 95, 14863-8.
11. Heyer LJ, Kruglyak S, Yooseph S. (1999) Genome Res. 9, 1106-15.
12. Zhang MQ. (1999) Genome Res. 9, 681-8

13. Perou C.M., Jeffrey S.S., Van de Run J., Rees C.A., Eisen M.A., Ross D.T., Pergamenschikov A., Williams C.F., Zhu S.X., Lee J.C.F., Lashkar D., Shalon D., Brown P.O. and Botstein D. (1999) Proc Natl Acad Sci U S A 96, 9212-17.
14. Gordon MK, Marchant JK, Foley JW, Igoe F, Gibney EP, Nah HD, Barembaum M, Myers JC, Rodriguez E, Dublet B, van der Rest M, Linsenmayer TF, Upholt WB, Birk DE (1999) Matrix Biol. 18, 481-6.