Gene expression profiles of di-n-butyl phthalate in normal human mammary epithelial cells

Detailed Data Collection Methods

Cell Culture

Primary normal human mammary epithelial cells (NHMECs) were derived from tissues salvaged at reduction mammoplasty, and obtained through the Cooperative Human Tissue Network (National Cancer Institute and National Disease Research Interchange). Development and characterization of cell strains was achieved using standard methods.17 Cells were grown in MEGM media (Clonetics, Cambrex, Pittsburgh, PA) at 37°C and 5% CO_2 [1].

DBP Treatment

Treatment was performed on cells at passage six and 70% confluency. Preliminary studies analyzed a range of DBP concentrations (1-100 µM) and time points (0-12 hr) and showed maximum effect of DBP on p53 expression with minimal toxicity at 10 hr with a final concentration of 1 µM. Cells were treated by diluting the stock DBP/acetone mixture in media and adding this solution to aspirated cells, allowing even exposure to all cells. Acetone (0.01%) alone was used as a vehicle control. All treatments were performed in triplicate. At the end of the treatment period, cells were removed for RNA isolation. Cell viability was determined by Trypan Blue exclusion assay.

Microarray Analysis

Microarray analysis was performed in triplicate using the U133A high-density oligonucleotide microarrays (Affymetrix™, Santa Clara, CA) as described previously [2]. Array analysis was performed using the Data Mining Tool version 3.0 (Affymetrix) with gene function determined from NetAffx (Affymetrix). All array data were first sorted for genes found to be present on at least one array under investigation, bringing the total number of genes to view to 13,072. From these genes, each cell strain was investigated for genes that are increased or decreased with exposure to DBP. These lists were then cross-referenced for all cell strains to determine which genes are affected by exposure in all cell strains, or only in cell strains with the same p53 haplotype. Data analysis was all performed using Self-Organizing Map Clustering with the filters [Threshold (Min = 20 ; Max = 20000); Row Variation (Max/Min = 3 and Max – Min = 100); Row Normalization (Mean = 0 and Variance = 1)] and parameters [Rows (3); Columns (3); Epochs (50); Seeds (1); Initialization (Random Vectors); and Neighborhood (Bubble)] set as per company recommendations.

Real-Time Polymerase Chain Reaction Analysis (RT-PCR)

cDNA synthesized from each sample as in the Affymetrix analysis (Invitrogen) was used m a one-step RT-PCR analysis reaction. Analysis was performed in duplicate on the ABI 7700 cycler, with the SYBR Green Master Mix (ABI). Primers were designed using Primer Express® (ABI) to yield unique fragments for each gene under study. Reacdons were set up following recommended protocols using 100 pmol of each primer (SigmaGenosys) and approximately 60 ng template per reaction. Reactions were performed in duplicate for each sample for 40 cycles (95°C/15 s denaturing step; 60°C/1 min annealing/extension step). Fold change was determined based on average cycle threshold (C_T) values for all duplicates and converted to signal log ratio.

References:

1] Gwinn MR et al. J Env. Pathol., Toxicol. & Oncol. 26:51-61, 2007.
2] Gwinn MR et al. ENV, Hlth. Perspcts. 113: 1046-1051, 2005.