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Environmental hydrocarbons have been implicated as significant risk factors in human atherosclerotic vascular disease. Using benzo(a)pyrene (BaP) as a model compound, this laboratory has shown that interference with gene transcription is a critical event in the induction of proliferative (i.e. atherogenic) phenotypes in vascular smooth muscle cells (vSMCs). The complexity of the cellular response elicited by BaP requires characterization of interactive gene networks involved in the atherogenic response. To achieve this objective, vSMCs isolated from the thoracic aorta of adult C57BL/6J were established in serial culture and used as a model to evaluate the atherogenic response to BaP. G0 synchronized cultures were released into growth by addition of fetal bovine serum in the presence of DMSO (control) or 3  $\mu$ M BaP for 8 hr, a regimen known to modulate vSMC phenotypes *in vitro*. Control and treated mRNAs were isolated and labeled with Cy3 and Cy5, respectively, and hybridized to custom-made mouse cDNA microarrays of 960 genes spotted in triplicate. Array intensities were evaluated as prescribed by GeneSpring<sup>®</sup> software to identify differentially expressed genes. Over 150 genes were altered in vSMCs by BaP treatment. Genes coding for RAS, cholesterol-regulated CR36 protein, several heat shock proteins, retinoic acid repressible protein, and insulin-like growth factor 1 receptor were significantly upregulated. Other genes increased by atherogen treatment included peroxiredoxin, cofilin, bromodomain PHD finger transcription factor, TIF1 beta, GTP-binding protein, G protein gamma-2 subunit, recA /RAD51, and BAF53a. These results confirm previous findings and implicate genes involved in ras signaling, oxidative stress, DNA repair, and chromatin remodeling in the atherogenic response elicited by BaP. (Supported by NIH grants ES 04849 and ES09106. CDJ is a postdoctoral fellow in NIEHS training grant ES 07273).

#### 42 IMMUNOMODULATORY EFFECTS OF 2, 3, 7, 8-TETRACHLORODIBENZO-P-DIOXIN IN LUNG CELLS AND/OR TISSUE.

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Interferon (IFN) can play a role in development of primary tumor and shape tumorigenicity. Previous results using real-time RT-PCR and microarray analysis of human peripheral lung airway epithelial cells (HPL1A), showed repression of several IFN regulated genes and induction of IFN regulatory factor 4 (IRF4) when treated with 2, 3, 7, 8-tetrachlorodibenzo-p-dioxin (TCDD). We hypothesize that TCDD alters the response of HPL1A cells to interferon. Since IFN-gamma is known to inhibit cell proliferation, cells were treated with 1X10<sup>4</sup> units/ml for 24 hours plus or minus TCDD (10nM) pretreatment. An aqueous soluble tetrazolium/formazan assay was used to detect cell growth changes. Up to 30 nM concentrations, TCDD did not cause any cell growth changes compared to cells given vehicle (FBS) only. By comparison, treatment with INF-gamma caused a 28 % decrease in cell growth ( $P < 0.001$ ). Pre-treatment with TCDD (10nM), reduced this decrease in cell growth caused by INF-gamma by approximately 50 % ( $P < 0.001$ ). Our next objective was to do a cross-species comparison looking at *in vivo* gene expression in rat lung. We sought to determine how TCDD treatment affected interferon regulatory factor 4 (IRF4) and myxovirus (influenza) resistance 1, homolog of murine interferon-inducible protein p78 (MX1). Sprague-Dawley rats were treated by oral gavage twice weekly. Control animals were given vehicle (corn oil) only, and the treated group consisted of a subacute treatment (350 ng/kg twice a week). Using RNA isolated from rat lung tissue, we analyzed IRF4 and MX1 expression by real-time RT-PCR. We found that IRF4 was induced after 5 weeks TCDD treatment, while MX1 was suppressed. This inverse gene relationship was the trend of expression we previously found in the HPL1A cell line. Overall, this data suggest a novel role for TCDD in immunomodulation in human lung cells that appears to be conserved in rats

#### 43 MATRIX-DEPENDENT ALTERATIONS IN REL PROTEIN EXPRESSION AND NF-KAPPAB ACTIVITY AFTER OXIDATIVE INJURY.

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Repeated cycles of chemical injury by allylamine induce proliferative (i.e. atherogenic) vascular smooth muscle cell phenotypes in male rats. Activated smooth muscle cells display matrix-specific changes in proliferation that correlate with NF-kappaB binding activity. Injured cells exhibit a proliferative advantage when seeded on plastic, fibronectin, or laminin, but not collagen. These cells also display altered

patterns of osteopontin expression, secretion, and cleavage, coupled with changes in the expression of several integrin subunits. Integrin-mediated increases in NF-kappaB activity may occur as a result of altered expression profiles of NF-kappaB constituent proteins. To test this hypothesis, the present studies examined changes in Rel protein expression in populations of control and oxidatively stressed cells following repeated cycles of allylamine injury. Sprague-Dawley rats (175-180 g) were gavaged once daily with allylamine (70 mg/kg) for 20 consecutive days. Vascular smooth muscle cells were isolated by enzymatic digestion, and cells maintained in serial culture for up to 25 passages. Western analysis demonstrated that Rel-A protein levels decreased in allylamine cells seeded on plastic, with concomitant appearance of a novel 50kDa immunoreactive protein. Furthermore, p50 protein levels increased in allylamine cells seeded on plastic, but not on collagen, fibronectin, or laminin. Seeding of allylamine cells on plastic, but not collagen, fibronectin, or laminin reduced Rel-B protein levels relative to controls. These results suggest that changes in Rel protein expression may contribute to the expression and/or maintenance of allylamine-induced atherogenic phenotypes. (This work was supported in part by NIH grants HL62539 and ES09106).

#### 44 TOXICOGENOMICS: FINGERPRINTING TOXIC COMPOUNDS USING GENE EXPRESSION PROFILES.

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In the recent past there has been considerable interest in linking the modulation of gene expression to the toxicity of drugs. Toxicogenomics is considered a valuable tool for the understanding of underlying mechanisms of toxicity and for the early prediction of toxic liabilities in toxicological studies. It is expected that gene expression profiles will generate fingerprints typical of classes of toxicants, as well as provide new and sensitive toxicity markers. In this paper we established and investigated the effect of five hepatotoxic compounds with a similar mechanism of toxicity on gene expression profiles in male Wistar rats. The so-called "direct acting compounds" tested in this study were Thioacetamide, Bromobenzene, CCl<sub>4</sub>, dichlorobenzene and hydrazine. The gene expression in the liver was evaluated using Affymetrix GeneChip<sup>®</sup> microarrays. Genes regulated by at least 4 of these compounds were considered characteristic for these toxins. This "direct acting fingerprint" was then compared with the modulation of the same genes caused by four steatotic compounds (Tetracycline, Amiodarone, Doxycycline and Amineptine) and by 1, 4-DCB, a non-toxic regioisomer of the "direct acting" compound 1, 2-DCB. The results presented in this paper show that gene expression profiles can be used to distinguish "direct acting" compounds from other types of compounds such as the non-toxic compound 1, 4-DCB and the tested steatotic compounds. Furthermore, the assessment of the modulation of gene expression in two experiments performed independently with the same compound showed good reproducibility of the gene expression profiles. In conclusion, gene expression profiles in liver after exposure to hepatotoxins are reproducible and characteristic of classes of toxins. Gene expression fingerprints allow different types of toxins to be distinguished from each other and from non-toxic compounds.

#### 45 COMPOUND PROFILING USING CDNA ARRAY BASED DIFFERENTIAL GENE EXPRESSION OF HUMAN AND RAT HEPATOCYTES.

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Compound profiling strategies are of great interest in the process of developing new pharmaceuticals. Combinatorial chemistry and high throughput screening technologies are flooding the development pipelines with possible candidates and efficient and reliable tools are required to properly rank them. Target validation and early predictive toxicogenomics are areas which can be especially forwarded through microarray applications. We have started to set up a ToxSAYS<sup>™</sup> database, where we are determining the gene expression of more than 1,000 well-defined human and rat ortholog genes in response to treatment with the 150 most frequently prescribed drugs and a whole range of chemicals with known toxicological effects. For the development of this database, we investigate gene expression profiles in primary rat and human hepatocytes, and in tissues and organs from GLP-compliant four-week toxicity studies. The selected genes are involved in Toxicology-relevant cell activities like apoptosis, cell cycling, proliferation, DNA damage/repair, inflammation, oxidative stress, transport and metabolism. For each gene 200-400 bp cDNA fragments are rigidly selected to avoid cross reactions between high homologous gene families. Starting with a set of toxic compounds e.g., Aroclor 1254, Allyl alcohol, Galactosamine, Concanavaline, Acetaminophen and Thioacetamide we demonstrate the induction of distinct gene expression profiles by each of these