was drastically changed in the presence of 10 nM TPA after 24-hrs exposure. Among C/EBPs, C/EBPα and C/EBPε were slightly affected by arsenite, but their activation were suppressed by TPA after 24-hrs exposure. NF-κB was constitutively activated by serum free conditions in U937 cells and this activation was suppressed by arsenite. These results indicate that arsenite and TPA interact with key regulators by different pathway in monocytic differentiation of U937 cells.

1165 IDENTIFICATION OF MOUSE SLC39A8 AS THE TRANSPORTER RESPONSIBLE FOR CADMIUM-INDUCED TOXICITY IN THE TESTIS.

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Testicular necrosis is a sensitive endpoint for cadmium (Cd++, Cd) toxicity across all species tested. Resistance to Cd-induced testicular damage is a recessive trait assigned to the Cdm locus on mouse chromosome 3. We first narrowed the Cdm-gene-containing region to 880 kb. SNP analysis of this region from two sensitive and two resistant inbred strains demonstrated a 400-kb haplotype block consistent with the Cd-induced toxicity phenotype; in this region is the Slc39a8 gene encoding a member of the solute-carrier superfamily. Slc39a8 encodes SLC39A8(ZIP8), whose homologs in plant and yeast are putative zinc transporters. We show here that ZIP8 expression in cultured mouse fetal fibroblasts leads to a >10-fold increase in the rate of intracellular Cd influx and accumulation and a 30-fold increase in sensitivity to Cd-induced cell death. The complete ZIP8 mRNA and intron-exon splice junctions have no nucleotide differences between two sensitive and two resistant strains of mice; using situ hybridization we found that ZIP8 mRNA is prominent in the vascular endothelial cells of the testis of the sensitive strains of mice, but absent in these cells of resistant strains. Slc39a8 is therefore the Cdm gene, defining sensitivity to Cd toxicity specifically in vascular endothelial cells of the testis.

1166 ROLE OF EUKARYOTIC TRANSLATION INITIATION FACTOR 4E (EIF4E) IN CADMIUM-INDUCED CYTOTOXICITY AND CELL DEATH.

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The role of eukaryotic translation initiation factor 4E (eIF4E), in cadmium-induced cytotoxicity and cell death was investigated. Exposure of human cell lines HCT15, PLC/PR/5, HeLa and Chang, to cadmium chloride (Cd) resulted in a dose-dependent toxicity and death. Western blot analysis of the cells demonstrated a significant inhibition of eIF4E gene (protein) in response to Cd exposure. Whether the inhibition of eIF4E was responsible for the observed toxicity and death was studied by silencing the cellular expression of eIF4E gene by employing a small interfering RNA (SiRNA) specifically targeting the eIF4E gene. The SiRNA-mediated silencing of eIF4E gene expression resulted in significant cytotoxicity and cell death suggesting that the cytotoxicity and cell death noticed among the Cd-treated cells were probably due to the chemical-induced inhibition of eIF4E gene expression. Transgenic Chinese hamster ovary cell lines overexpressing eIF4E were resistant to Cd-induced cytotoxicity and cell death. Results of Western blot analysis and immunoprecipitation experiments demonstrated a significant induction of ubiquitination of eIF4E in the Cd treated cells. Pre-exposure of cells to proteasome inhibitors blocked the Cd-induced inhibition of eIF4E gene expression as well as the resulting cytotoxicity and cell death. Furthermore, exposure of cells to Cd resulted in a significant inhibition of expression of the cell cycle and growth regulating gene, cyclin D1. Transfection of cells with SiRNA specifically targeting eIF4E gene expression also resulted in a significant inhibition of cyclin D1 gene expression suggesting that the observed inhibition of cyclin D1 gene in the Cd-treated cells is most likely mediated through inhibition of eIF4E gene. Taken together, our results demonstrate that the exposure of cells to cadmium chloride resulted in cytotoxicity and cell death due to the enhanced ubiquitination and proteolysis and the consequent inhibition of eIF4E gene expression leading to diminished cellular level of critical genes such as cyclin D1.

1167 MECHANISMS OF ARSENITE-STIMULATED HEMEOXYGENASE-1 UPREGULATION IN HUMAN KERATINOCYTES.

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Hemeoxygenase-1 (HO-1) is the rate-limiting enzyme involved in heme catabolism. It is an oxidative stress responsive gene upregulated by various physiological and exogenous stimuli including heme, ultraviolet irradiation, heat shock, inflammatory cytokines, heavy metals and arsenite. HO-1 has many stress-activated recognition sites in the promoter region of its gene. These include NF-κB, heme response elements, antioxidant response elements and activator protein 1 (AP-1). Arsenic is a known dermatotoxin and chronic exposure has been associated with increased incidence of keratinocytic tumors. The mechanism of arsenic-mediated skin carcinogenesis is not well-understood, but activation of mitogen-activated protein kinases (MAPKs) and generation of reactive oxygen species (ROS) may contribute to tumor promotion and progression. We have reported that arsenite (AsIII)-dependent activation of ERK, but not p38, is dependent upon EGF receptor activity. In this study we investigated the potential contributions of ROS generation and AP-1 activation to AsIII-dependent regulation of HO-1 in HaCat cells, a spontaneously immortalized human keratinocyte cell line. Both EGF and AsIII induced ROS as observed via dihydroethidium (DHE) staining and fluorescence microscopy. Western blotting showed arsenite-induced sustained upregulation of HO-1 in a time-dependent (0-72h) and concentration-dependent (3–30μM) manner. Inhibition of EGF receptor, MEK I/II, and p38 activation moderately reduced HO-1 expression, but none completely abrogated the arsenite-induced response suggesting that the signaling proteins (EGF receptor, ERK and p38) were necessary but not sufficient for AsIII-induced HO-1 upregulation. Inhibition of Src-family kinases also slightly reduced HO-1 induction indicating a small contribution to the observed induction. The superoxide scavenger MnTMPyP also partially decreased the arsenite-induced expression of HO-1. These results suggest that both the stress-activated and EGF receptor pathway are involved in regulation of HO-1 expression in response to arsenite in keratinocytes.

1168 MERCURY, CADMIUM, ZINC, AND ARSENITE INHIBIT PAX3 DNA BINDING VIA THE PAIRED DOMAIN.

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Mercury, cadmium, zinc, and arsenite are common metallic environmental contaminants that have high affinities for endogenous thiols, such as glutathione and protein thiols. Pax3 is a murine transcription factor that is involved in a number of developmental processes, including neural tube closure. Using the Splotch mouse model, it has been shown that Pax3 haploinsufficiency confers added sensitivity to neural tube defects (NTDs) induced by arsenite. Previous studies have also shown that cysteine residues in the Pax3 paired domain (PD) are required for binding to PD-specific DNA target sites and that such binding is reduced or blocked entirely by pro-oxidants. The current study was undertaken to test whether the presence of an environmentally relevant metal is capable of abrogating Pax3 binding to a PD-specific DNA target, the e5 segment from the *Drosophila* even-skipped gene. Electrophoretic mobility shift assays (EMSAs) were performed with 32P labelled e5 in the presence of 2mM glutathione, a cellularly relevant concentration. Mercuric chloride, cadmium chloride, zinc sulfate, and sodium arsenite were each able to dose-dependently inhibit Pax3 DNA binding with EC50s of 10, 50, 60, and 450 uM, respectively. Thus far, this work demonstrates that, like the sensitivity to pro-oxidants, Pax3 binding to DNA targets via the PD is sensitive to the presence of thiophilic metals. Experiments are underway to examine the effect of the one-electron redox cycling metals, copper and iron, in the presence and absence of the one-electron reductant, ascorbate, to determine to what extent redox chemistry plays a role in mediating Pax3 DNA-binding inhibition. We are also examining the effect of GSH:GSSG ratios in the presence and absence of thiophilic or redox cycling metals to determine the role of redox environment in the inhibition of Pax3 DNA binding via the PD.

1169 NFκB MEDIATES ZINC-INDUCED COX-2 EXPRESSION IN HUMAN BRONCHIAL EPITHELIAL CELLS.

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Upregulation of COX-2 expression is a pivotal event in inflammatory reactions induced by a variety of stimuli. Divalent zinc (Zn2+) component has recently been implicated as a causative agent in airway inflammation induced by exposure to ambient particulate matter. Our recent studies have shown that Zn2+ exposure increases COX-2 expression through both transcriptional and posttranscriptional mechanisms in human bronchial epithelial cells. This study aims to determine whether the transcription factor nuclear factor κB (NFκB) mediates Zn2+-induced COX-2 mRNA expression in these cells. Exposure to Zn2+ had a minimal effect on IκBα breakdown and nuclear translocation of NFκB. However, Zn2+ exposure caused a time-dependent phosphorylation of NFκB (p65), a component of the NFκB transcription factor, suggesting a transactivation mechanism activated by Zn2+ exposure. Consistent with this, exposure of cells to Zn2+ resulted in a time-dependent increase in NFκB reporter activity. Inclusion of human specific NFκB

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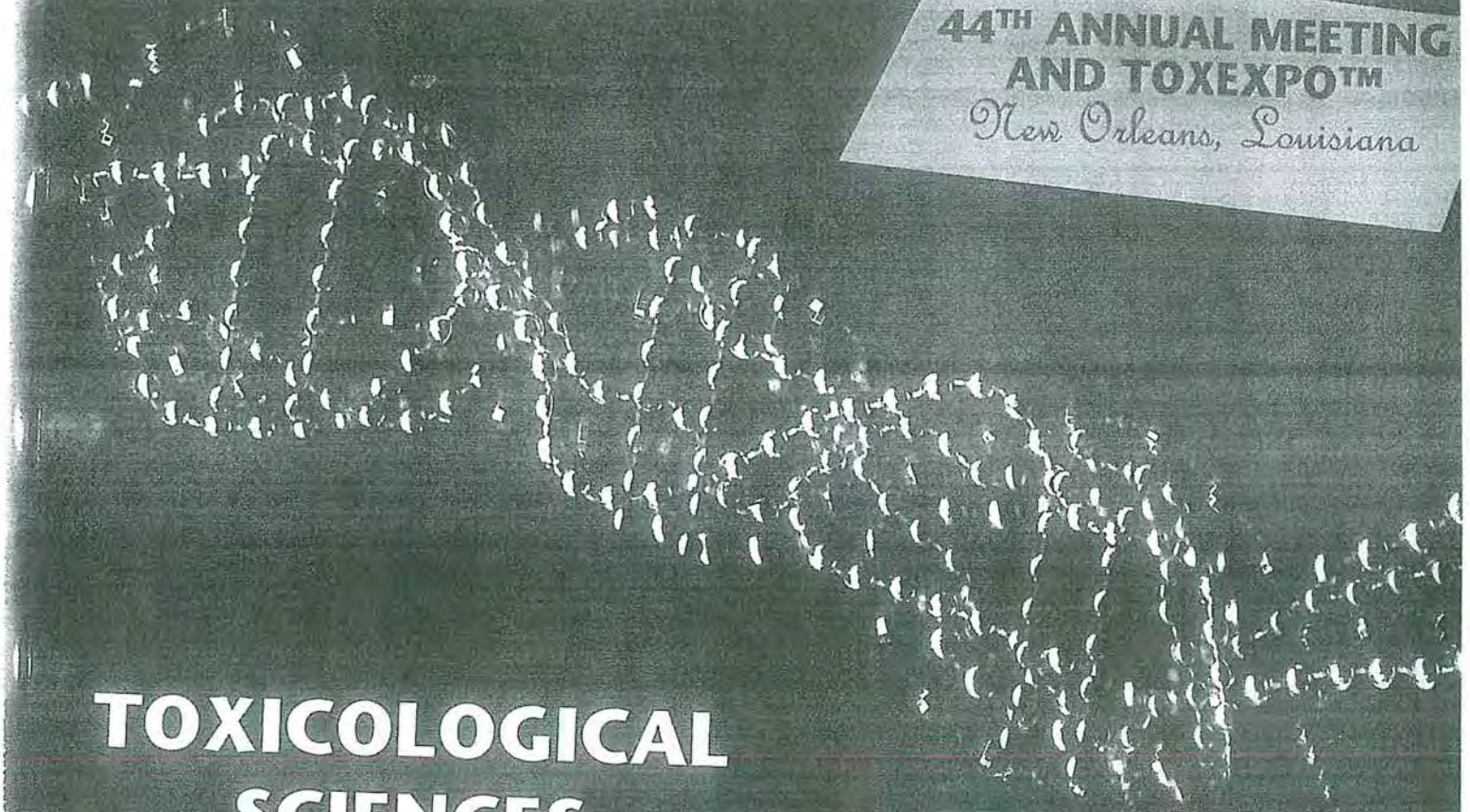

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