pressed GST Ya catalyzed detoxification capacity of the cells at the mRNA and activity levels. The three metals enhanced the expression of heme oxygenase-1 (HO-1) in a dose-dependent manner suggesting that HO-1 is involved in the modulation of AHR-regulated gene expression by heavy metals. The ability of metals to alter the capacity of AHR ligands to induce the bioactivating phase I and the detoxifying phase II enzymes will influence the carcinogenicity and mutagenicity of the AHR ligands.

265 DIFFERENTIAL EFFECTS OF HEAVY METALS ON ARYL HYDROCARON RECEPTOR-REGULATED GENES.

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Both simultaneous and sequential exposure to heavy metals and aryl hydrocarbon receptor (AHR) ligands potentially occur in human populations, yet there have been relatively few studies of combined effects of heavy metals and AHR ligands on AHR-regulated genes. The aim of this work was to investigate the potential effects of heavy metals, particularly mercury (Hg^{2+}), lead (Pb^{2+}), and copper (Cu^{2+}), on the constitutive and the inducible expression of AHR-regulated genes; cytochrome P450 1a1 (cyp1a1), NAD(P)H:quinone oxidoreductase (QOR) and glutathione S-transferase Ya; (GST Ya) at the activity and mRNA levels. For this purpose, murine hepatoma hepa 1c1c7 wild-type cells were incubated with increasing concentrations of Hg^{2+} (2.5, 5 and 10 μM), Pb^{2+} (10, 25 and 100 μM), and Cu^{2+} (1, 10 and 100 μM) alone or with the AHR-ligand, 2, 3, 7, 8-tetrachlorodibenzo-*p*-dioxin (0.1 nM), 3-methylcholanthrene (0.25 μM), β-naphthoflavone (10 μM), or Benzo[a]pyrene (1 μM). Our results clearly showed that metals alone did not significantly alter the cyp1a1 activity, whereas a significant reduction in AHR ligand-mediated induction of cyp1a1 activity was observed by all metals in a concentration-dependent manner. Interestingly, the decrease in cyp1a1 activity was associated with an increase in cyp1a1 mRNA level. With respect to QOR, the activity was strongly increased dose-dependently by all metals in the absence or presence of an AHR-ligand, with the exception of Cu^{2+} which significantly decreased the activity. Changes in QOR at the mRNA levels were in accordance with the activity results. Differently, GST Ya activity was significantly increased by Cu^{2+} and Pb^{2+} and inhibited by Hg^{2+}. All metals significantly increased the expression of stress protein, heme oxygenase-1 (HO-1). We conclude that heavy metals differentially modulate the constitutive and the inducible expression of AHR-regulated genes. Chronic exposure of heavy metals/AHR-ligands mixture would be less effective in inducing AHR-mediated toxicity than exposure to metals or AHR-ligand alone.

266 ARSENITE INITIATES AH RECEPTOR-INDEPENDENT REPRESSION OF CYP1A1 INDUCTION BY TCDD.

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Arsenite (As^{3+}) alters the expression and function of several drug-metabolizing enzymes, including cytochrome P450 1A1 (CYP1A1). In addition, it has been shown that *CYP1A1* expression can be regulated in a cell cycle dependent manner. Interestingly, As^{3+} interrupts cell cycle control by initiating G2/M arrest. To examine the impact of As^{3+} on TCDD initiated induction of CYP1A1, HepG2 cells were treated with sub-cytotoxic (0.5 μM to 50 μM) doses of As^{3+}. Exposure of HepG2 cells to TCDD and As^{3+} revealed an As^{3+} dose-dependent increase in G2/M phase arrest. Vinblastine causes G2/M arrest and was shown to decrease TCDD-induced *CYP1A1* gene expression. Examination of the catalytic activity of CYP1A1 as measured by EROD assay in HepG2 cells demonstrated that As^{3+} led to a dose-dependent decrease in TCDD-induced EROD activity. This reduction correlated with a comparable reduction in CYP1A1 as determined by Western blot analysis. The addition of As^{3+} to microsomal preparations generated from TCDD treated cells did not influence EROD activity, suggesting that inhibition occurs upstream of translation. This was verified by quantitative analysis of CYP1A1 mRNA by real-time RT-PCR. The lowest concentration of As^{3+} (0.5 μM) resulted in a 61% reduction in TCDD-induced CYP1A1 mRNA. Using HepG2 cells that express the human *CYP1A1*-luciferase reporter gene, it was demonstrated that As^{3+} did not alter TCDD induction of *CYP1A1*-luciferase. Gel shift analysis confirmed activation of the AhR and subsequent binding to the XRE following co-treatment with TCDD and As^{3+}. Combined, *CYP1A1* initiated transcription does not appear to be a target for the inhibitory properties of As^{3+}. These results indicate that G2/M phase arrest may be a factor in As^{3+} inhibition of TCDD initiated induction of CYP1A1. Moreover, the mechanism of inhibition does not impact on activation of the AhR. (Supported by USPHS grant ES10337).

267 ARSENIC-TRANSFORMED HUMAN PROSTATE EPITHELIAL CELLS SHOW CHANGES IN ANDROGEN METABOLISM AND ESTROGEN RECEPTOR EXPRESSION.

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Inorganic arsenic (As) is an important environmental toxicant, with natural and industrial sources. Human As exposure is linked to cancers in various tissues including the prostate. However, the molecular mechanisms of As carcinogenesis are still poorly defined. Steroid hormones play a critical role in stimulating the growth and differentiation of normal prostate epithelial cells, as well as in the initial growth of prostate cancer cells. Furthermore, genes involved in the prostatic steroid signaling pathways have been implicated in cancer development. Variation in the androgen or estrogen receptor genes, as well as in the steroid metabolizing enzymes, such as, the steroid 5α-reductase, have been linked to prostate cancer causation and progression. We have developed an *in vitro* model of As-induced prostate cancer by inducing malignant transformation of the immortalized, non-tumorigenic human prostate epithelial cell line RWPE-1 by chronic As exposure. To help understand the molecular mechanisms underlying As-induced malignant transformation, we performed gene expression comparisons between these chronic As transformed prostate epithelial cells (designated CAsE-PE) and control RWPE-1 cells. RT-PCR and western blot were used to examine expression of ER-α and ER-β. Our studies revealed an increase in ER-β (1.6-fold) and a decrease in ER-α (45%) transcript in As transformed cells compared to control. However, the correlate receptor protein levels were significantly elevated from 20 and 100% in CAsE-PE cells. 5α-reductase transcript, which encodes for the enzyme that converts testosterone to its active metabolite, DHT, was markedly increased in CAsE-PE cells. These data suggest altered expression of steroid receptors and altered steroid metabolism occurs in CAsE-PE cells. These changes in steroid metabolism and sex steroid signaling pathways may be associated with As-induced malignant transformation and prostate cancer.

268 EXPOSURE TO SOLUBLE NICKEL ALTERS IRON-MEDIATED GENE TRANSCRIPTION AND ENZYME ACTIVITY IN A549 CELLS.

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Nickel compounds have been identified as human carcinogens/cocarcinogens using both *in vivo* studies and epidemiological evaluations. Nickel is known to stabilize the hypoxia inducible factor-1α (HIF-1α) protein, which is thought to be important in tumor progression. Since nickel affects HIF-1α, which is controlled by an iron dependent enzyme, we designed *in vitro* experiments to look at the effect of nickel on iron dependent processes in A549 cells. Using the Affymetrix Gene Chip, we identified several iron-regulated genes that were altered in reponse to nickel treatment. The mRNA levels of transferrin receptor and GADD45, both of which are increased in response to low iron levels, were substantially increased in response to nickel. In addition, the transcription of many HIF-1α-dependent genes was also increased. Nickel chloride was also able to block the induction of ferritin by ferrous iron. Atomic absorption was then performed to see if nickel had an effect on total iron levels in A549 cells. The levels of total iron were significantly lowered in response to 1mM nickel chloride. Furthermore, the enzyme activity of several iron dependent enzymes, including prolyl hydroxylase and aconitase, was significantly lowered when cells were exposed to 1mM nickel chloride. Surprisingly, when we treated a cell extract with concentrations of nickel chloride as low as 10 uM, prolyl hydroxylase activity was decreased. In sharp contrast, when we treated purified aconitase enzyme with concentrations of nickel chloride as high as 2mM, we observed no decline in enzyme activity. The prolyl hydroxylase enzymes that control the stability of HIF-1α appear to be very sensitive to very low concentrations of nickel. Iron dependent processes are a requirement for life and interference with these processes may be involved in nickel-induced carcinogenesis. These data may give new insight into the mechanisms of nickel induced carcinogenesis, as well as, contribute important information for the treatment and prevention of occupational diseases.

269 EUKARYOTIC TRANSLATION INITIATION FACTOR 4E (EIF4E) IS A CELLULAR TARGET FOR CADMIUM TOXICITY.

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Cadmium, to which large numbers of people are occupationally and otherwise exposed, has been categorized as a human carcinogen by the International Agency for Research on Cancer (IARC). Several theories have been proposed to account for the

mechanisms potentially responsible for cadmium toxicity and carcinogenesis. Recently, our laboratory has demonstrated a novel mechanism for cadmium carcinogenesis that involves the upregulation of translation initiation factor 3 and translation elongation factor-1 delta. Presently, we have investigated whether the translational proto-oncogene, eukaryotic initiation factor 4E (eIF4E) is a cellular target for cadmium toxicity and carcinogenesis. Four different human cell lines: HCT-15 (colorectal adenocarcinoma), PLC/PRF/5 (hepatocellular carcinoma), HeLa (adenocarcinoma) and Chang (likely derived from HeLa cells) cells, were exposed to 30 μM cadmium chloride for time intervals up to 24-hours and the expression level of eIF4E was determined by Western blot analysis using an antibody against human eIF4E. Exposure to 30 μM cadmium chloride resulted in significant cytotoxicity and cell death in all four cell lines tested. Furthermore, exposure to cadmium chloride resulted in significant inhibition of eIF4E in all cell lines, and the highest inhibition was noticed following the 24-hours exposure to cadmium chloride. The significance of cadmium chloride-mediated inhibition of eIF4E was further investigated by silencing the expression of eIF4E by employing small interfering RNA (SiRNA) specifically targeting eIF4E. The SiRNA mediated silencing of eIF4E resulted in significant cell death. Our results thus suggest that eIF4E is a cellular target for cadmium toxicity and that the cadmium-induced cytotoxicity and cell death may be due to the inhibition of eIF4E.

270 LEAD IS MITOGENIC TO WTHBF-6 CELLS, BUT LEAD CHROMATE (LC) INDUCES CELL CYCLE ARREST.

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Hexavalent chromium (Cr(VI)) is a human lung carcinogen of significant public health concern. The particulate Cr(VI) compounds are more potent carcinogens than soluble ones. Preliminary reports suggest that LC, a prototypical particulate Cr(VI) compound, partially dissolves to produce ionic lead (Pb) and Cr and that while the Cr ions are damaging DNA, the lead ions are stimulating cell growth. We found that Pb induced cell growth in WTHBF-6 cells: 250, 500, 1000, 2000 uM lead glutamate (LG) induced 40, 54, 85, and 142% increases in the mitotic index. However, LC did not stimulate cell growth: 0.5, 1.0, and 5.0 ug/cm^2 reduced the mitotic index by 52.9, 52.9, and 92.35% respectively and metaphases were absent at 10.0 and 50.0 ug/cm^2. These same LC concentrations induced prolonged growth arrest. Furthermore, LC-induced arrest occurred at intracellular lead concentrations much lower than those found for growth stimulatory levels of ionic lead. We found that soluble sodium chromate (SC) also inhibited growth and the concentrations of Cr inside the cell after both LC and SC were equivalent. Further analysis of the cell cycle showed that 0.5, 1.0, 5.0 and 10.0 ug/cm^2 LC increased the percentage of cells in S-phase by 9.7, 24.3, 83.5, and 104% respectively. These increases coincided with a drop in the percentage of cells in G1 phase. SC also induced accumulation of cells in S-phase at moderate concentrations. Yet only 2000 uM LG, resulted in S and/or G2/M arrest. These results indicate that Cr, not Pb, is responsible for cell growth effects associated with LC. This work was supported by NIEHS grant 1R01 ES 10838-01 (J.P.W.).

271 MERCURY MODULATES CELL CYCLE PROGRESSION IN HUMAN LIVER CARCINOMA CELLS THOUGH INDUCTION OF C-FOS, CYCLIN-A, AND CYCLIN-D EXPRESSION, AND REPRESSION OF GADD153.

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Exposure to mercury has been associated with a significant number of adverse health effects on various organ systems, and cancer in some cases. Its toxicity has been attributed to its high affinity to sulfhydryl groups of proteins. However, little is known regarding the molecular mechanisms by which mercury exerts its toxicity, mitogenesis, and/or carcinogenesis. Recent studies in our laboratory have shown that mercury is cytotoxic and transcriptionally activates stress genes in human liver carcinoma (HepG2)cells. We hypothesized that mercury-induced expression of stress proteins and cyclins may play a role in the molecular events leading to toxicity and tumorigenesis. To test this hypothesis, we performed the MTT-assay for cell *via*bility, and the Western Blot and densitometric analyses to assess the expression of specific cellular proteins including c-fos, GADD153, cyclin-A, and cyclin-D. Data obtained from the MTT-assay indicated a strong dose-response relationship with respect to mercury toxicity. The LD50 was computed to be 3.5 +/- 0.6 μg/mL upon 48 hrs of exposure. Western blot and densitometric analyses showed c-fos, cyclin-A, and cyclin-D expressions to increase in a dose-dependent manner within the dose-range of 0-3 μg/mL; showing a peak induction at 3 μg/mL and a sharp drop at 4 μg/mL, probably due to cell death at higher levels of mercury exposure. No statistically significant differences ($p > 0.05$) were found in GADD153 expression between control and mercury-treated cells. Taken together, these findings indicate that mercury is cytotoxic, and has the potential to modulate cell cycle control proteins in HepG2 cells. Induction of c-fos/cyclin-D and cyclin-A expression indicates its stimulation of cell cycle progression, respectively through the G1/S and G2/M transitions. The ramifications of these findings to our understanding of merucy-induced toxicity and tumorigenesis are important. [Research supported by NIH-RCMI Grant No. 1G12RR13459, and DoA-MACERAC Grant No. DACA-42-02-C-0057].

272 URANIUM IS CYTOTOXIC AND GENOTOXIC TO HUMAN LUNG CELLS.

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Maine has some of the highest rates in the nation for bladder and lung cancer. It is also a state that relies heavily on well water, and many of these wells are contaminated with uranium. In addition, uranium has also recently become a specific concern for the health of our soldiers, as well as for those exposed through fragments left behind in the environment, because of the heavy use of depleted uranium in military armor and munitions. Uranium potentially has both chemotoxic and radiotoxic effects. Previous investigations have largely focused on the radiotoxic effects of uranium and its decay products; much less is known about uraniums potential chemical toxicity. One major chemotoxic effect that has been characterized is nephrotoxicity, and there is reason to consider uranium a potential bladder carcinogen because chronic exposures have been shown to be carcinogenic. Uranium has also been associated with human lung cancer. As a first step to understanding the potential carcinogenic mechanisms of uranium, we treated WTHBF-6 cells, a human lung fibroblast cell line with concentrations ranging from 1- 200 uM uranium acetate for 24 h and determined its cytotoxic and genotoxic effects. We found that uranium was moderately cytotoxic over this range with 89.9%, 57.2%, and 40.6% relative survival at 25, 50 and 100 uM. We also found that uranium was clastogenic inducing multiple observable lesions in chromatid structure. At concentrations of 50, 100 and 200uM, 3%, 4% and 19% damage of mitophases was noted in preliminary data. The spectrum of chromosome damage included chromosome breaks, which are consistent with the types of karyotypic alterations seen in many neoplasms. Future research is aimed at understanding the genotoxic mechanisms involved in uranium-induced neoplastic transformation

273 INTERFERON-α INDUCTION OF METALLOTHIONEIN IN RAT LIVER IS NOT LINKED TO INTERLEUKIN-1, -6 OR TUMOR NECROSIS FACTOR-α.

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Synthesis of metallothionein (MT) is induced by interferon-α (IFN-α) *in vitro* and *in vivo*. In addition, IFN-α promotes redistribution of zinc (Zn) from the plasma to the liver in mice. However, it is not clear if IFN-α induces hepatic MT synthesis directly or indirectly *via* liberation of other cytokines. In order to address this issue, we determined hepatic MT levels, Zn concentration in plasma, liver and urine, and plasma levels interleukin-1 (IL-1), interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α) in rats following intramuscular injection of human IFN-α (1.5 X 106 UI/m2). Animals were housed in metabolic cages and sacrificed at various times after IFN-α administration. Zn concentrations in serum, urine and hepatic tissue were determined by atomic absorption spectrophotometry. MT protein was measured using the MT silver saturation method and Western-blot analysis. Plasma levels of IL-1, IL-6, and TNF-α were determined using an ELISA method. Hepatic MT levels began to increase at 2 h following IFN-α administration and reached maximum levels at 12 h post-treatment. IFN-α also produced biphasic increases in hepatic Zn, with levels peaking at 2 h, the time-point when MT levels are first increased, and again at 18 h. Concurrently, there were decreases in plasma Zn levels at these time points, suggesting IFN-α-induced movement of Zn from the blood to hepatic tissue. The decrease in plasma zinc was not due to increased excretion since urinary Zn levels were unaffected following IFN-α treatment. IFN-α administration had no effect on plasma IL-1, IL-6 and TNF-α levels. These results show that IFN-α promotes the increase of hepatic MT levels and plasma/liver redistribution directly, without IL-1, IL-6 or TNF-α participation

274 PROLYL HYDROXYLASES AS TARGETS FOR CARCINOGENIC NICKEL.

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Nickel is a well established human carcinogen, however mechanisms of its carcinogenicity are not understood. Recently, we performed a microarray analysis of cells exposed to soluble nickel and observed strong induction of hypoxia-inducible

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