leucine zipper transcription factor, plays a central role in the regulation of expression of antioxidant enzymes through binding to antioxidant or electrophile response elements in the promoter regions of the target genes. Thus, the effects of acute inorganic arsenite (As^{3+}) exposure on Nrf2 expression and translocation, as well as expression of Nrf2 related genes, were studied in HaCaT cells, an immortalized human keratinocyte cell line. When HaCaT cells were exposed to As^{3+} (0, 2.5 or 10 μM) and fractionated, Nrf2 protein accumulated in the nuclear fraction in a time and dose-dependent fashion which reached a peak at 6 hr. Total cellular Nrf2 protein and cytosolic Nrf2 increased in a similar fashion with As^{3+} exposure but both reached peaks at 12 hr. These results suggest As^{3+} up-regulates Nrf2 expression and induces Nrf2 translocation from the cytosol to the nucleus. Additional study showed the expression of the manganese superoxide dismutase (MnSOD) gene, a possible target of Nrf2, increased after As^{3+} exposure. The levels of MnSOD RNA were correlated with Nrf2 protein levels in nuclear extract, suggesting Nrf2 mediated MnSOD up-regulation by As^{3+}. When HaCaT cells were pre-treated with Tiron, a scavenger of superoxide radical, As^{3+}-induced increases in Nrf2 levels in all cellular fractions were suppressed, indicating As^{3+}-induced superoxide formation is a possible regulator of Nrf2. These results suggest that As^{3+} alters Nrf2 expression and cellular localization, and activates expression of an Nrf2-related gene (MnSOD) in HaCaT cells. These actions appear related to oxygen radical formation.

1307 P53 SIGNALING PATHWAY INVOLVED IN ARSENITE-INDUCED HOS CELL TRANSFORMATION AND ITS PREVENTION BY CAFFEIC ACID PHENETHYL ESTER(CAPE).

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Chronic arsenic exposure is of concern mainly because of its carcinogenicity, but its mechanism is not well understood. Many studies have focused on the expression of the p53 gene in response to acute exposure to arsenite, but very little data concerning the role of p53 in response to chronic arsenic exposure are available. HOS (human osteoblast-like) cell transformation assay was used to mimic chronic exposure to arsenic and explore the mechanism of the p53 signaling pathway using genearrays. After HOS cells were continuously exposed to low concentrations of arsenite (NaAsO2, 0.3 uM) for 8 weeks, transformed HOS (As-HOS) cells were cloned on agar. mRNA expression related to p53 signal pathway in As-HOS cells was significantly down regulated as compared to parental HOS cells. The average fold decreases for p53, mdm2, gadd45, gadd45b, p21, pig8, bax, and TRPM2 were 5.4, 10.5, 32.2, 8.1, 5.9, 7.1, 6.2, and 16.3, respectively. When HOS cells were incubated together with arsenite and CAPE (0.5 uM) for 8 weeks, the cell transformation was suppressed and the expression of above genes increased 3.1, 3.1, 7.7, 3.1, 7.3, 2.5, 1.7 and 12.2 fold, respectively, compared to those expressed by As-HOS cells. Since we also found that CAPE induces As-HOS cell death by apoptosis but causes no damage to parental HOS cells, we conclude that the deficiency in p53 and its target genes, such as gadd45 (growth arrest gene), p21 (cyclin-dependent kinase inhibitor), bax (apoptosis gene), mdm2 (p53 binding protein), Pig8 (p53 interaction gene), and PRPM2 (apoptosis gene), caused by arsenite prevents the HOS cells from entering cell cycle arrest or apoptosis, and leads to cell transformation instead. Since the lower expression of p53 mRNA made As-HOS cells more vulnerable to an anticancer agent CAPE, enhancing the p53 signaling pathway might be helpful in preventing arsenite-induced cell transformation. [Supported in part by NIH grants, ES10344, ES 00260, and CA 37858]

1308 EFFECT OF CADMIUM ON P53 AND MITOGEN-ACTIVATED PROTEIN KINASES IN A MURINE MACROPHAGE CELL LINE: RELATION TO APOPTOSIS.

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Cadmium (Cd) is a persistent metal pollutant prevalent in the environment. It is immunotoxic *in vivo* and induces apoptosis in many cultured cells. P53 and mitogen-activated protein kinases (MAPKs) are important regulators of apoptosis; however, the Cd-modulated p53 and MAPKs signaling mechanisms leading to cellular toxicity on macrophages have not been investigated. Present study was designed to determine the impact of cadmium on cell proliferation, cell cycle and apoptosis, and to investigate the possible involvement of p53 and MAPKs signaling pathways in J774A.1 murine macrophages. Cd inhibited cell proliferation *via* cell cycle arrest and induced apoptosis in a dose-dependent manner. Cd at 20 μM markedly increased cells in G2/M and hypodiploid sub-G1 phases of the cell cycle suggesting cycle arrest and cell death. Treatment with Cd at 20 and 50 μM induced phosphorylation of extracellular signal-regulated kinase (ERK), but did not alter p53 mRNA expression or the activation of p38 MAPK and c-Jun N-terminal MAPK. ERK inhibitor, PD98059, suppressed Cd-induced ERK activation. Inhibition of ERK suppressed DNA synthesis and had an additive effect with Cd-inhibited proliferation suggesting that Cd-induced ERK activity is not responsible for the G2/M arrest and subsequent inhibition of cell proliferation. Instead, the increase in ERK activation in cells treated with Cd may reflect a stress response. Pretreatment with PD98059 increased Cd-induced cytotoxicity suggesting that ERK activation may be a survival response in the present cell system. Cycloheximide, an inhibitor of protein synthesis, did not alter Cd-induced cytotoxicity indicating that J774A.1 cell death by Cd is independent of *de novo* protein synthesis including p53. Results suggested that p53 is not involved in Cd-induced cell cycle arrest and apoptosis in J774A.1 cells. ERK activation by Cd is not related to decreased proliferation of macrophages but may play a protective role against Cd-induced cytotoxicity.

1309 MITOGEN AND STRESS SIGNAL TRANSDUCTION PATHWAYS CONTRIBUTE TO SODIUM ARSENITE-INDUCED CYCLOOXYGENASE-2 EXPRESSION IN NORMAL HUMAN EPIDERMAL KERATINOCYTES.

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Arsenic is an atypical human skin carcinogen whose mode of action is not defined. Based on evidence that arsenic influences inflammatory events that are involved in skin carcinogenesis and dermatotoxicity, this study addressed the ability of sodium arsenite to regulate the pro-inflammatory enzyme cyclooxygenase-2 (COX-2) in normal human epidermal keratinocytes (NHEK). It was hypothesized that arsenite regulates COX-2 expression in NHEK; an effect dependent on specific mitogen- and stress-related signal transduction pathways. NHEK were exposed to sodium arsenite in a dose- and time-dependent manner and COX-2 expression/activity, DNA synthesis, and mitogen activated protein kinase (MAPK) phosphorylation quantified. Specific inhibitors of p42/44 and p38 MAPK pathways were used to examine the contribution of mitogen and stress signaling to arsenite-induced COX-2 expression. Non-cytotoxic concentrations of arsenite (≤ 5μM) elevated COX-2 expression in a dose-dependent manner at the gene and protein level, as well as increased prostaglandin E2 (PGE_2) secretion; a functional measure of COX-2 activity. Arsenite also initiated an acute and delayed increase in the phosphorylated/activated form of p42/44 MAPK, but did not stimulate p38 MAPK phosphorylation. Pharmacological inhibition of mitogen-activated protein kinase kinase (MEK) using the inhibitor PD98059 partially attenuated arsenite-induced *COX-2* mRNA expression whereas the p38 MAPK inhibitor SB202190 had minimal effect on the induction of *COX-2*. In contrast, elevation of COX-2 protein by arsenite is reduced by both PD98059 and SB202190 treatment. These results demonstrated that sodium arsenite modulates COX-2 in NHEK at the transcriptional, translational, and functional level; effects dependent in part on MAPK signaling. Because COX-2 plays a critical role in skin cancer, this enzyme and/or the pathways that regulate its expression could be key components involved in arsenic skin carcinogenesis.

1310 ACTIVATION OF ERK SIGNALING PATHWAY AND AP-1 IN UROTSA CELLS BY INORGANIC AND METHYLATED TRIVALENT ARSENICALS.

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Arsenic is classified as a human carcinogen. The molecular mechanism by which arsenic induces cancer is not well understood. In humans, inorganic arsenic (iAs) undergoes oxidative methylation, yielding trivalent and pentavalent mono- and dimethylated species. Recent studies have shown that trivalent methylated arsenicals are more cytotoxic, genotoxic and more potent enzyme inhibitors than trivalent iAs, arsenite (iAsIII). Mono- and dimethylated trivalent arsenicals were detected in the urine of individuals chronically exposed to iAs. It is generally accepted that transactivation of the transcription factor AP-1 is required for tumor promotion. In this study, we examined signaling pathways that are responsible for AP-1 activation in SV40-immortalized normal human urothelial (UROtsa) cells exposed to iAsIII, monomethylarsine oxide (MAsIII) or iododimethylarsine (DMAsIII). One-hour exposures to 0.1 to 5.0 μM iAsIII, MAsIII or DMAsIII significantly increased DNA-binding activity of AP-1. The EMSA/supershift and Western blot analyses showed that p-c-Jun and Fra-1 were the major AP-1 constituents. MAsIII was the most potent activator of AP-1 followed by DMAsIII and iAsIII. The activation of AP-1 by trivalent arsenicals was associated with an increased phosphorylation (activation) of ERK1, 2 and ERK5. Pretreatment of UROtsa cells with PD98059 or U0126 (specific inhibitors of the MEK/ERK signaling pathway) completely suppressed phosphorylation of ERK1 and 2, but only partially decreased the phosphorylation of ERK5 in cells exposed to MAsIII or DMAsIII. Similarly, treatment with either inhibitor decreased, but not abolished the phosphorylation of c-Jun in exposed cells. Neither arsenical induced activation of JNK1, 2 or p38 under these exposure conditions. These results indicate that trivalent arsenicals induce AP-1 DNA-binding activity in UROtsa cells through ERK-mediated phosphorylation of c-Jun and that ERK5 may be an essential component of this mechanism.