1920 ALTERNATIVE PROMOTERS DETERMINE TISSUE-SPECIFIC EXPRESSION PROFILES OF HUMAN MICROSOMAL EPOXIDE HYDROLASE (EPHX1).

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Microsomal epoxide hydrolase (EPHX1) catalyzes hydration reactions that determine the cellular disposition of reactive epoxide derivatives derived from xenobiotic exposures. Whereas the previously defined human EPHX1 exon 1 sequence, E1, is derived from a gene promoter immediately proximal to exon 2 of the EPHX1 coding region. Remarkably, the E1 promoter directs expression only in the liver. We have identified an alternative EPHX1 promoter that is localized ~18.5 kb upstream of exon 2. Northern hybridizations demonstrated that the E1b promoter functions as the primary driver of EPHX1 expression in human extrahepatic tissues. Using a rolling circle amplification-RACE technique, together with EST database analyses, we have identified 2 distinct EPHX1 transcripts in extrahepatic tissues and cell lines that are derived from the E1b promoter region. We term the transcripts E1b and E1b'. The E1b transcript includes a noncoding 46 bp exon 1 in its 5'-untranslated region. In contrast, the length of the E1b' 5'-UTR is extended, and includes 2 upstream AUG codons together with high GC content. Analyses of the human E1b gene promoter region indicated the presence of multiple transposable elements, including Alu repetitive elements of the AluSp, AluSx, AluJo and AluYa5 classifications. The AluYa5 elements are relatively recent in evolutionary history, and their presence in the EPHX1 promoter was associated with significant reduction in EPHX1 transcriptional activity following transfection experiments conducted in transformed human cell lines derived from multiple tissues, including kidney 293A, lung A549, hepatoma HepG2, and breast cancer MCF7. Further investigations are underway to assess the regulatory features of this promoter that direct its transcriptional activity in human tissues.

1921 ACQUIRED ANDROGEN INDEPENDENCE DURING CADMIUM-INDUCED MALIGNANT TRANSFORMATION OF HUMAN PROSTATE EPITHELIAL CELLS.

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Early human prostate cancer (PCa) is typically androgen-dependent but can progress to an aggressive androgen-independent (AI) state that is frequently lethal. AI growth often involves changes in the androgen receptor (AR). Cadmium (Cd) is a possible prostate carcinogen potentially linked to aggressive PCa. Thus, acquisition of AI growth was studied in normal prostate epithelial RWPE-1 cells malignantly transformed by chronic low-level Cd exposure (CTPE cells). CTPE cells were hyperproliferative compared to control, and androgens or antiandrogens had little effect on this proliferation. Prostate specific antigen (PSA) was markedly increased while the tumor suppressor gene *p27* was reduced in CTPE cells, changes typical in AI PCa. AR levels were similar in control and CTPE cells but androgen actually induced less AR-related gene expression in CTPE cells. Thus, this AI growth is unrelated to changes in AR levels or sensitivity. AI growth can involve estrogen signaling via the estrogen receptor (ER). In fact, after exposure to estradiol (E2) CTPE cell proliferation greatly increased while control cells showed little response. ERα transcript was elevated in CTPE cells, while ERβ transcript was distinctly lower. Antiestrogen treatment nearly abolished E2-induced CTPE cell growth. CTPE cells had increased 5α-aromatase (5-αA), which metabolizes testosterone to estrogen. Disruption of IGF signaling is frequently found in AI PCa and IGF expression is frequently controlled by estrogen. Although basal levels were similar, E2 markedly (6.5-fold) increased in IGF-1 in CTPE cells but not in control, indicating IGF-1 is a key factor in E2-induced CTPE cell proliferation. Thus, Cd-induced malignant transformation of human prostate cells precipitates AI growth, unrelated to AR expression or activity. Increased ER and 5α-A expression and IGF-1 responsiveness suggests estrogen signaling may be critical to Cd-induced acquired AI growth, which fortifies their emerging role in PCa development and progression.

1922 EUKARYOTIC TRANSLATION ELONGATION FACTOR 1δ OVEREXPRESSING CELLS ARE RESISTANT TO CADMIUM-INDUCED TOXICITY AND APOPTOSIS.

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The role of Eukaryotic translation elongation factor 1δ (eEF1δ) in cadmium-induced cytotoxicity and apoptosis was investigated. Transgenic cell line overexpressing the eEF1δ gene were generated by transfecting HeLa cells with the expression vector pcDNA 3.1 containing human eEF1δ cDNA. Stable transfectant cell line was developed using blasticidin (5mg/mL medium). Expression of eEF1δ in the transgenic cell line was determined by real-time PCR and Western blot analysis. Cell line exhibiting 2 fold (eEF1δ4) and 3 fold (eEF1δ11) increases in eEF1δ expression were developed. The vector alone transfected cell line served as the control. The transgenic cell line exhibited enhanced cell proliferation, overexpression of eEF1δ, eIF4E and eEF2 genes compared with the control cell line. Cadmium-induced cytotoxicity was significantly lower in the eEF1δ overexpressing cell line compared with the control. Cadmium induced apoptosis was also studied using the control and eEF1δ overexpressing cell lines. The eEF1δ overexpressing cell line exhibited significantly higher resistance to cadmium-induced apoptosis compared with the control cell line. Real-time PCR analysis of eEF1δ overexpressing cell line exposed to cadmium showed significant induction of anti-apoptotic genes like Bad and Bcl-2, with a concurrent decrease in the expression of the pro-apoptotic gene, p53 compared with the control cell line. In summary, the eEF1δ gene overexpressing cell line exhibited significant resistance to cadmium-induced cytotoxicity and apoptosis.

1923 TOXICOGENOMIC ANALYSIS OF CADMIUM RESPONSIVE TRANSCRIPTION IN *CAENORHABDITIS ELEGANS* REVEALS NOVEL GENES AND PATHWAYS INVOLVED IN HEAVY METAL RESISTANCE.

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The transition metal cadmium is a known human carcinogen, and exposure to this metal is associated with a variety of human diseases. Although individuals are continuously exposed to this metal, pathologic effects are prevented by intracellular defense and repair systems. In the present study, we used *C. elegans* as a model system to identify (a) genes that are induced by a sub-lethal level of cadmium; (b) biological pathways and functions that are affected by cadmium, and (c) genes that are induced by cadmium to protect nematodes against metal toxicity. The global transcriptional changes after 4h and 24h exposure to a sub-lethal concentration of cadmium (100μM) were measured using *C. elegans* whole genome arrays. Gene ontology analysis indicated that *C. elegans* metabolism and transport pathways were significantly enriched with differentially expressed genes following both 4h and 24h exposure, while proteolysis and fatty acid metabolism pathways were affected only in 24h exposure. The relationship between cadmium-induced expression changes and protection against cadmium toxicity was investigated by quantifying growth and reproduction after RNA interference. We identified a list of cadmium-inducible genes that were related to cadmium sensitivity in *C. elegans* and many of them have catalytic or binding activities. Protein interaction analysis also revealed that *kel-8*, which is a signaling molecule expressed in neurons, was required in the defense response against cadmium toxicity. Further, the protective effect of *kel-8* depends on normal function of *mek-1*, suggesting that a novel pathway is involved in the metal response. We are also applying the information obtained from *C. elegans* to the mammalian system by studying the homologue of a novel cadmium-responsive *C. elegans* gene in human HEPG2 cells.

1924 LOSS OF GLUTAMATE-CYSTEINE LIGASE MODIFIER SUBUNIT SENSITIZES MICE TO CHRONIC CADMIUM TOXICITY.

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High concentrations of Cd cause toxicity in several organs including liver, lung, bone and kidney. Reduced glutathione (GSH) is one of the cell's major defenses against oxidative stress and has also been shown to possess a protective role against Cd damage, acting as a "first line of defense" in both animals and cultured cells. Glutamate-cysteine ligase modifier subunit (GCLM) has the overall effect of increasing the efficiency of GSH synthesis. *Gclm(-/-)* knockout mice generated in our laboratory show GSH levels that are 15% (liver) and 25-30% (kidney) of that in *Gclm(+/+)* wild-type littermates. Numerous studies have demonstrated Cd as being a toxic metal that causes oxidative stress. Cd toxicity, in the context of chronic GSH depletion, has not been studied-most likely because of complications in interpreting data from animals chronically treated with chemicals that deplete GSH, such as buthionine sulfoximine. To examine the role of chronic GSH depletion in chronic Cd toxicity, we compared *Gclm(-/-)* mice and WT littermates. The effects of repeated subcutaneous injections (6 per week) of $CdCl_2$ at a dose of 10 μmol/kg vs isotonic saline (0.9% NaCl) given to both genotypes were compared. This dose of Cd is not acutely lethal, even to sensitive strains. All mice were given 1 mL of water by gavage once a week, urine was collected for 2 h in metabolic cages, and blood collected via saphenous vein. Increases in weight loss, plasma ALT and AST, blood urea nitrogen, and urinary *N*-acetyl-β-*D*-glucosaminidase (NAG), suggesting liver and kidney failure preceding mortality, occurred in both genotypes. Fifty percent mortality with $CdCl_2$ treatment occurred in 28 days for *Gclm(-/-)* mice vs 40 days

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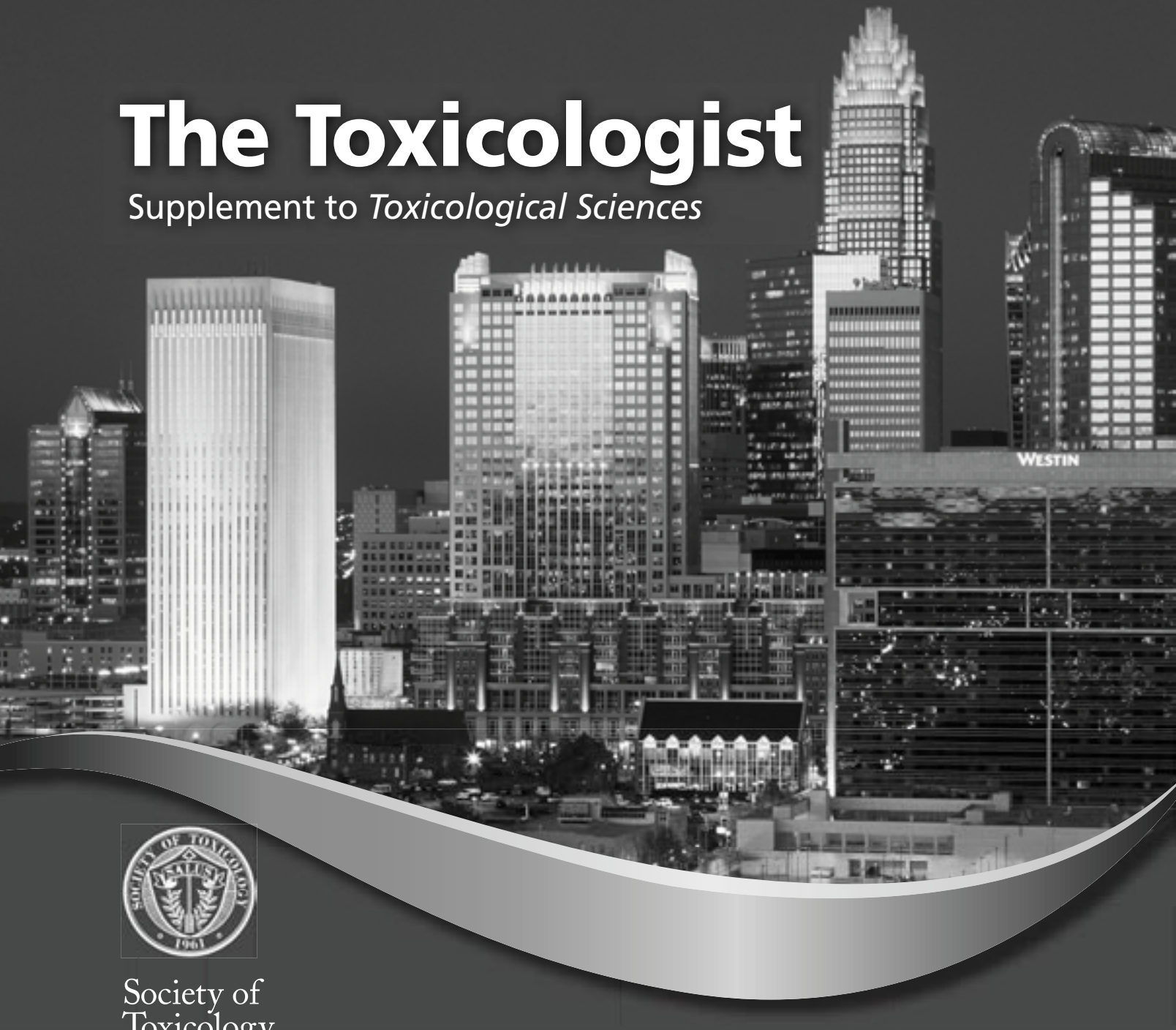

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Preface

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An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 449.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 480.

The abstracts are reproduced as accepted by the Program Committee of the Society of Toxicology and appear in numerical sequence.

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