spleen, cultured and stimulated *in vitro* with concanavalin A, in the presence or absence of $HgCl_2$. There were clear age-dependent and tissue-specific differences in both proliferation (measured by ^{3}H-thymidine uptake) and cytokine production (interferon-γ and interleukin-4). Lymph node cells from 10 day pups produced more IFN-γ than cells from 7 day pups; adult cells produced the least amount of IFN-γ. In pups, thymus cells from 10 day pups produced more IFN-γ than cells from 7 day pups. Cells from spleen and lymph node cells from the youngest mice produced the most IL-4; cells from pups of both ages produced more IL-4 than adults. There were no age-related differences in lymph node cell proliferative response; however, adult splenocytes had higher proliferative responses compared to 10 day pups. $HgCl_2$ at this low dose did not affect proliferative responses or IL4 production in any cells at any age. In contrast, adult splenocytes were more sensitive to $HgCl_2$ in terms of inhibiting IFN-γ production. These results indicate the importance of developmental stage in studying immunotoxicity. This information is particularly relevant to understanding Hg toxicity, since perinatal exposures are of great public health concern. Supported by NIH-Fogarty and a Merit Award from the VA to EKS.

150 STEM CELL BIOLOGY IN TOXICOLOGY.

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Stem cells can divide asymmetrically to give rise to a daughter that is committed to terminally differentiate and another daughter that can maintain "stemness". In addition, they have the potential to divide symmetrically to expand their numbers. Toti-potent stem cells give rise to pluri-potent stem cells, which, in turn can give rise to committed progenitor cells. Since the demonstration that pluripotent stem cells, pre-derived from embryonic but also from adult tissue, the question arises, "Is there a differential sensitivity to toxicants between pluripotent stem cells, its committed progenitors and the terminally-differentiated daughters"? The stem cell theory of carcinogenesis implies that these cells are the target cells for carcinogenic initiation. Until recently, little attention has been given to the potential differential sensitivities of the stem cell family. In the usual molecular and biochemical studies (including that of the DNA microarray technology) of toxic effects on organs/organisms or of comparative studies of normal *versus* diseased tissues, DNA, RNA and proteins are extracted from the whole tissue, which contains primarily progenitor and terminally differentiated and only a few stem cells. If a toxicant differentially affects the cells of the stem cell lineage, the examination of the combined DNA lesions, expressed genes and protein products of all of these cell types could mask the critical events if they occurred only in the "target"or stem cells. Human breast epithelial, human kidney, human neuronal and human pancreatic stem cells do not have functional gap junctional intercellular communication (GJIC). Exposure to c-AMP-inducing agents triggers GJIC and differentiation. Agents that block GJIC appear to block differentiation. The use of epigenetic toxicants on human stem cell systems could provide new approaches to understanding their contribution to in vivo toxic effects, since their reactions to these toxicants could be very different from differentiated cells with drug metabolizing systems.

151 CADMIUM-INDUCED CELL TRANSFORMATION AND TUMORIGENESIS ARE ASSOCIATED WITH TRANSCRIPTIONAL ACTIVATION OF C-FOS, C-JUN AND C-MYC PROTO-ONCOGENES: ROLE OF CELLULAR CALCIUM AND REACTIVE OXYGEN SPECIES.

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The molecular mechanisms of cell transformation induced by cadmium (Cd) were studied using BALB/c-3T3 cell transformation and nude mouse tumorigenesis models. BALB/c-3T3 cells transformed with CdCl2 were subcutaneously injected into nude mice to develop tumors and the cell lines derived from these tumors were used in this study. The proto-oncogenes c-fos, c-jun and c-myc were overexpressed in the cell lines. Tumor cells stained with fluorescent dyes specific for reactive oxygen species (ROS) revealed that these cells possessed markedly higher levels of superoxide anion and hydrogen peroxide compared with the nontransformed cells. Similarly, the intracellular calcium (Ca) level was higher in the tumor cells compared with the nontransformed cells. Overexpression of the proto-oncogenes in these cells was blocked by superoxide dismutase, catalase and BAPTA/AM confirming that the overexpression of the proto-oncogenes in the tumor cells required elevated levels of ROS and Ca. Inhibitors specific for transcription, PKC and MAP kinase also blocked the overexpression of the proto-oncogenes in the tumor cells. Exposure of the nontransformed BALB/c-3T3 cells to CdCl2 for 1 hr caused elevated intracellular levels of superoxide anion, hydrogen peroxide and Ca with corresponding increases in the expression levels of c-fos, c-jun and c-myc. As in the case of the tumor cells, treating the non-transformed cells with the various modulators prior to their exposure to CdCl2 resulted in inhibition in the expression of the proto-oncogenes. Based on these data, we conclude that the Cd-induced overexpression of cellular proto-oncogenes is mediated by the elevation of intracellular levels of superoxide anion, hydrogen peroxide and Ca. Further, the Cd-induced overexpression of the proto-oncogenes is dependent on transcriptional activation as well as on pathways involving PKC and MAP kinase.

152 INDUCTION OF P53 GENE EXPRESSION IN MCF-7 BREAST CANCER CELLS BY 17β-ESTRADIOL IS MEDIATED BY PROTEIN TYROSINE KINASE ACTIVATION OF AN NF-κB/CTF-1 COMPLEX.

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The tumor suppressor gene p53 plays an important role in regulation of cell growth and repair of DNA damage and p53 mutations are observed in multiple tumor types. Estrogen receptor (ER)-positive MCF-7 human breast cancer cells express wild-type p53 which is induced by 17β-estradiol (E2). We have investigated the mechanism of hormonal regulation of p53 gene expression and analysis of the p53 gene promoter has identified a minimal E2-responsive region at -106 to -40. This 67 bp region of the promoter does not bind ER but binds many other nuclear transcription factors including CTF-1/YY1, NF-Y, Sp1, Sp3 and E-box proteins, and the results of transcriptional activation and gel mobility shift assays show that motifs that bind CTF-1 and NF-κB proteins are required for hormone-responsiveness. The pathway for transactivation of p53 is novel and requires induction of protein tyrosine kinases by E2 and direct kinase-dependent phosphorylation of the p65 subunit of NF-κB. CTF-1 physically interacts with p65 but is not directly affected by E2 suggesting the CTF-1 acts primarily as a DNA-bound coactivator of p65. Thus, p53 tumor suppressor gene has now been identified as a downstream target for E2-activated protein tyrosine kinases in MCF-7 cells, and this response is mediated through an NF-κB-CTF-1 complex. (Supported by NIH ES09253 and ES09106.)

153 VASCULAR ENDOTHELIAL GROWTH FACTOR (VEGF) IS INDUCED BY 17β-ESTRADIOL (E2) IN ZR-75 HUMAN BREAST CANCER CELL LINE.

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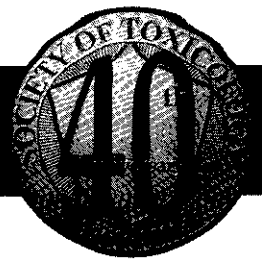
VEGF is a critical angiogenic factor expressed by tissues in response to hypoxia, hypoglycemia, growth factors and steroid hormones. Normal physiological processes of wound healing and the menstrual cycle require VEGF and VEGF receptor expression for efficient neovascularization. Transformed cells selectively increase VEGF gene expression to form new blood vessels, effectively increasing nutrient and oxygen supplies and providing a route for tumor cells to metastasize. ZR-75 breast cancer cells express VEGF and cells transiently transfected with human VEGF gene promoter-luciferase reporter constructs and human estrogen receptor α (ERα) expression plasmid increase luciferase activity in response to E2 treatment. The VEGF gene promoter does not contain "classical" estrogen responsive elements (ERE); however, numerous G/GC-rich sites that bind transcription factors Sp1 and Sp3 have been identified in this promoter. Previous studies in this laboratory have demonstrated that genes such as c-*fos*, adenosine deaminase, IGFBP-4 and RARα1 are E2-responsive in breast cancer cell lines through interactions of ERα with Sp1 protein, in which the ERα/Sp1 complex enhances gene expression through binding G/GC-rich elements. 5' Deletion analysis of the VEGF gene promoter has revealed a GC-rich -66/-47 Sp1-binding element that is necessary and sufficient for E2-increased luciferase activity. Based on results of previous studies and our analysis of the VEGF gene promoter, transcriptional activation of the VEGF gene by E2 in ZR-75 breast cancer cells may dependent on the ERα/Sp1 protein complex or other GC-rich binding protein interactions with the VEGF gene promoter motif at -66 to -47. (Supported by NIH CA76636 and ES09106.)



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Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, platform, poster discussion, workshop, roundtable, and poster sessions of the 40th Annual Meeting of the Society of Toxicology, held at the Moscone Convention Center, San Francisco, California, March 25–29, 2001.

An alphabetical Author Index, cross referencing the corresponding abstract number(s), begins on page 451.

The issue also contains a Keyword Index (by subject or chemical) of all the presentations, beginning on page 479.

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

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