1642 THE BENZENE METABOLITES HYDROQUINONE AND BENZOQUINONE INCREASE C-MYB ACTIVITY IN HD3 CELLS: AN INSIGHT INTO BENZENE MEDIATED LEUKEMOGENESIS.

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Benzene, a ubiquitous environmental toxicant, has been proposed to lead to carcinogenesis. While the mechanism behind benzene mediated leukemogenesis remains unknown, it is generally accepted that benzene exerts its toxicity after being metabolized in the body by cytochrome P450s. During blood cell development, the transcription factor c-Myb plays an important role such that mice lacking expression of this protein die *in utero* and are anaemic. Furthermore, overexpression of c-Myb prevents differentiation of induced erythrocytes. Therefore we hypothesize that c-Myb may be involved in benzene-mediated leukemogenesis. To investigate this hypothesis, cells were transiently transfected with a luciferase reporter gene containing the chicken mim-1 promoter and then exposed to 0-100 μM of hydroquinone and benzoquinone for up to 24 hours. Our results show that the benzene metabolites benzoquinone and hydroquinone can significantly increase c-Myb activity at 24 hours but not at 6 hours in a dose dependant fashion. Previous studies indicate that exposure to catechol, but not benzene or phenol, can increase c-Myb activity. In light of these past results, this study supports the hypothesis that benzene must be metabolized before mediating its toxicity and provides insight into benzene's actions on the c-Myb signalling pathway as a potential mechanism of leukemogenesis. (Support:CIHR and PREECAN)

1643 ACTIVATION OF DOWNSTREAM RAS EFFECTORS IN LUNG LESIONS FOLLOWING DOXYCYCLINE (DOX) REGULATED EXPRESSION OF MUTANT HUMAN KI-*ras* IN A BITRANSGENIC MOUSE MODEL.

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Previous studies from our laboratory reported the development of a bitransgenic mouse model that expresses the mutant human Ki-*ras*CYS[12] allele in alveolar type II and/or Clara cells in a DOX-regulated, lung-specific manner. When DOX was administered in the drinking water for up to 12 months, macroscopically visible lung lesions were first detected by 3 months, showing an increase in number and size during the course of DOX treatment. Microscopically, hyperplasia could be seen as early as 12 days and had progressed to adenomatous lesions by 6 months. Interestingly, none of the tumors progressed beyond the adenoma stage even after 12 months of DOX treatment. Withdrawl of DOX for 1 month resulted in a lack of proliferative lesions, suggesting tumor regression in the absence of Ki-*ras* expression. A 2-fold activation of both the RAS and RAL pathways in DOX-treated mice was detected by measuring the binding of tissue lysates to the Ras Binding Domain of Raf or Ral Binding Protein, respectively. Immunohistochemical analyses of the lung tissues demonstrated elevated phosphorlylation of downstream RAS effectors. Increased phosphorlyation of Erk, p90 ribosomal S6 kinase, ribosomal S6 protein, and the transcription factor CREB was detected with antibodies specific for phosphorylated residues of these proteins. The highest levels of activated signaling proteins were seen in bronchiolar epithelium, cells surrounding blood vessels, and the hyperplasic or adenomatous tissue. These results suggest that mutant RAS-mediated activation of the RAF-MEK-ERK and possibly the RAL pathways plays a critical role in the early stages of lung tumorigenesis. Continued studies with this *in vivo* model will allow the delineation of the role of these and other RAS effector pathways in the lung neoplastic process. (Supported by NCI grant CA91909 and CA91909-S1)

1644 OVEREXPRESSION OF PKC EPSILON IN THE MOUSE EPIDERMIS LEADS TO POLYMORPHONUCLEAR NEUTROPHIL INFILTRATION AND EPIDERMAL DESTRUCTION AFTER A SINGLE TOPICAL DMBA-TPA TREATMENT.

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We have previously reported that FVB/N transgenic mice which overexpress protein kinase C epsilon (PKCε) in basal epidermal cells and cells of the hair follicle developed papilloma-independent metastatic squamous cell carcinoma (mSCC) elicited by an initiation (7, 12-dimethylbenz[a]anthracene, DMBA) - promotion (12-O-tetradecanoylphorbol-13-acetate, TPA) protocol. The present studies were performed to determine early morphological events in PKCε transgenic mouse skin treated with DMBA/TPA. FVB/N wild type mice and PKCε transgenic mice were initiated with a single dose of 100 nmol DMBA, followed by a single topical application of 5 nmol TPA one week later. Dorsal skin samples were fixed for histological examination at 0, 12, 24, 48, 72, and 96 hrs after TPA treatment. At 12 and 24 hrs, polymorphonuclear neutrophils (PMNs) infiltrated into the epidermis in both wild type and PKCε transgenic mice, and the epidermal area involved by PMN infiltration was not significantly different. However, complete epidermal necrosis by 48 h was observed in PKCε transgenic mice, not in their wild type littermates. Epidermal cell regeneration was observed at 72 h post TPA treatment in PKCε transgenic mice. The regenerated skin was disordered and showed extensive hyperplasia and hyperkeratosis. In addition, lack of staining with the differentiation marker keratin 10 showed the regenerated epidermis to be poorly differentiated. These histological changes were not observed in PKCδ overexpressing transgenic or in wild type mice. These data suggested that a single TPA treatment of the dorsal skin of PKCε transgenic mice resulted in epidermal destruction by PMNs, followed by hyperproliferation of cells of the basal layer and hair follicle and resulting in a hyperplastic, poorly differentiated epidermis. Our results may provide insights into the mechanism of the development of papilloma independent mSCC in PKCε transgenic mice.

1645 ACTIVATION OF AP-1 AND PRO/ANTIOXIDANT STATUS IN SKIN OF AP-1 TRANSGENIC MICE DURING CANCER PROMOTION WITH CUMENE HYDROPEROXIDE.

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Organic peroxides, widely used in the chemical and pharmaceutical industries, can act as skin tumor promoters and cause epidermal hyperplasia. They are also known to trigger free radical generation. The present study evaluated the effect of cumene hydroperoxide (Cum-OOH) on the induction of activator protein-1 (AP-1), which is linked to the expression of genes regulating cell proliferation, growth, and transformation. Previously, we reported that topical exposure to Cum-OOH caused formation of free radicals and oxidative stress in skin of vitamin E deficient mice. In addition, *in vitro* studies found that exposure to Cum-OOH reduced levels of GSH in JB6 P^+ cells and caused the induction of AP-1. The present study used AP-1-luciferase reporter transgenic mice to identify whether exposure to Cum-OOH *in vivo* caused activation of AP-1, oxidative stress, depletion of antioxidants and tumor formation during two-stage carcinogenesis. Mice primed with dimethylbenz[α]anthracene (DMBA) were treated topically with Cum-OOH (82.6 μmol) or 12-O-tetradecanoylphorbol-13-acetate (TPA, 17 nmol) twice weekly for 20 weeks. Activation of AP-1 in skin was detected as early as 2 weeks following Cum-OOH and TPA exposures. Maximum AP-1 expression was detected 4 weeks post initiation with Cum-OOH or TPA. No AP-1 activation was found 19 weeks post initiation. Papilloma formation was observed in both the DMBA/TPA and DMBA/Cum-OOH exposed animals, while skin carcinomas were found only in the DMBA/Cum-OOH treated mice. A greater accumulation of peroxidative products (TBARS), inflammation, and decreased levels of GSH, vitamin E and total antioxidant reserves were also observed in the skin of DMBA/Cum-OOH exposed mice. These results suggest that Cum-OOH induced carcinogenesis is accompanied by increased AP-1 activation and changes in antioxidant status.

1646 REDUCTION OF COX-2 EXPRESSION IN THYROID FOLLICULAR EPITHELIAL CELLS DURING DHPN-INDUCED CARCINOGENESIS IN RATS.

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In thyroid follicular epithelial cells, constitutive expression of cyclooxygenase-2 (COX-2) has been demonstrated. To assess the role of COX-2 during rat thyroid carcinogenesis, we evaluated its expression in the proliferative lesions induced by goitrogens with or without *N*-bis(2-hydroxypropyl)nitrosamine (DHPN)-initiation. DHPN was subcutaneously injected once to 40 male F344 rats at age of 6-week. One week after the initiation, goitrogens such as propylthiouracil (PTU) and sulfadimethoxine (SDM) were administered in drinking water for 4 or 10 weeks, and then thyroid samples were collected for histopathology and immunohistochemistry for COX-2. Goitrogen alone groups were also placed. At week 4, multiple focal follicular cell hyperplasias and adenomas were frequently observed in DHPN + PTU and DHPN + SDM groups. In DHPN + SDM and SDM alone groups, severe inflammation with fibrosis in the thyroid capsule with migrated follicular epithelial cells into the capsule were also observed. At week 10, increased incidences and multiplicities of focal hyperplasias and adenomas in DHPN + PTU and DHPN + SDM groups were observed. In addition, invasive adenocarcinomas to capsule were also detected in 3 of 10 rats of the DHPN + SDM group. These carcinomas were suggested to originate in the focal hyperplasias and adenomas adja-

Society of Toxicology
43rd Annual Meeting
Baltimore, Maryland

THE TOXICOLOGIST

a supplement to
TOXICOLOGICAL SCIENCES

An Official Journal of the Society of Toxicology

Volume 78, Number S-1, March 2004

Preface

This issue of *The Toxicologist* is devoted to the abstracts of the presentations for the symposium, workshop, roundtable, platform and poster sessions of the 43rd Annual Meeting of the Society of Toxicology, held at the Baltimore Convention Center, Baltimore, Maryland, March 21-25, 2004.

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