plus NNAL-Gluc in smokers (n = 20; 20.9 ± 4.6 (8–30) cigarettes/day) and nonsmokers (n = 30). Average total NNAL excretion in smokers was 3242 ± 1954 (373–8058) pmol/d. Self-reported nonsmokers completed a questionnaire on ETS exposure over 1 week and wore personal nicotine samplers during this period, prior to urine sampling. Based on urinary cotinine excretion (cut off point 0.5 μmol/d), 1 subject appeared to be a misclassified smoker. Of the remaining 29 subjects, 12 had no detectable total NNAL (urinary cotinine: 9.3 ± 5.7 (5.1–20.2) nmol/d; personal nicotine: 0.15 ± 0.18 (0.05–0.52) $\mu g/m^3$), but 17 had an average excretion of 42.3 ± 44.9 (3.8–148.3) pmol/d (urinary cotinine: 78.1 ± 119.2 (5.8–459.4) nmol/d; personal nicotine: 0.99 ± 1.22 (0.11–4.07) $\mu g/m^3$). Self-reported exposure to ETS was 3.6 ± 6.5 (0–18) and 19.8 ± 27.2 (1–100) h/wk, respectively. For the 17 nonsmokers with detectable NNAL excretion, data correlated with both cotinine ($r = 0.58$, $p = 0.0276$) and nicotine ($r = 0.75$, $p = 0.0012$). No correlation was found between total NNAL and ETS-h ($r = 0.08$, $p = 0.7629$). The biological relevance of these data will be discussed.

#2276 Persistence of urinary metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) after smoking cessation. Hecht, S.S., Carmella, S.G., Koch, D., Chen, M., Jensen, J., Lando, H., and Hatsukami, D.K. *University of Minnesota Cancer Center, Minneapolis, MN 55455*

NNK is a tobacco-specific lung carcinogen and a likely causative agent for lung cancer in smokers. Lung cancer risk is known to persist for years after smoking cessation, but the mechanism has not been elucidated. The retention of lung carcinogens or their metabolites in smokers after cessation has not been previously examined. NNK is metabolized to its carbonyl reduction product NNAL and the corresponding glucuronide NNAL-Gluc, which can be quantified in human urine. In this study, we examined the excretion of NNAL and NNAL-Gluc after smoking cessation. Twenty-eight subjects were enrolled and randomized to treatment with nicotine patch or no patch; all subjects received counseling. Twenty-four h urine samples were collected at baseline, then 1, 3, 6, 10, 14, and 18 weeks after smoking cessation. They were analyzed for NNAL and NNAL-Gluc. Data for six subjects not using the patch indicate prolonged excretion of NNAL and NNAL-Gluc. Levels of NNAL plus NNAL-Gluc at baseline were 2.36 ± 1.62 pmol/ml urine. Setting baseline levels of NNAL plus NNAL-Gluc as 100%, amounts in urine were as follows: 25 ±10% (week 1); 12 ± 9% (week 3); 6 ± 3% (week 6); 6 ± 3% (week 10); 1.5 ± 1.3% (week 14). These data suggest that the lung carcinogen NNK or its metabolite NNAL is retained in a compartment of the smoker's body after smoking cessation. Supported by grant CA-44377 from NCI and P50 DA-09259 from NIDA.

#2277 Racial differences in sensitivity of platelets to anaphylactic aggregation in endometrial cancer patients. Ionov, I.D., Smolin, Yu. N., Sokolav, A.V., and Krasilova, M.D. *Orthopharm Medical Center, Leninsky pr. 123-4-63, Moscow 117513, Russia.*

Possible cause of racial differences in survival of women with endometrial cancer is considered. Two groups of white (9) and black (9) women suffering from endometrial cancer with no significant difference in the median duration of abnormal uterine bleeding, stage of disease, grade of differentiation, histologic type and myometrial invasion have been observed. Platelets were significantly more aggregable to platelet activating factor (PAF) in black group as compared to whites (p less than 0.01). We have previously suggested that sensitivity of blood coagulating system to PAF and other anaphylactic mediators can play role in tumor growth. Development of tumor is accompanied by local anaphylactic reactions against tumor antigens which lead to release of anaphylactic mediators near the antigens. Mediators (PAF primarily) induce the formation of microthrombi which «mask» antigens and in result protect tumor from immune damage furthering its growth (Ionov, 1986–91). Thus increased aggregation-inducing action of PAF in black patients with cancer can be the reason of poorer outcome.

#2278 Validation of biomarkers of benzene using HPLC-MS assay in a Chinese population with broad range of occupational benzene exposure. Melikian, A.A., Meng, M., Ni, J., Qu, Q., Chen, L.C., Cohen, B., Shore, R., Jin, X., Li, J., Yin, S., Mu, R., Li, Y. *American Health Foundation, Valhalla, NY 10595, New York University, NY 10987.*

Exposure to high doses of benzene is associated with aplastic anemia, leukemia, and lung cancer, and possibly also with Hodgkin's lymphoma and multiple myeloma. Health effects of low-dose exposure to benzene are not precisely known. To address this uncertainty, an HPLC-MS/MS assay had been developed and applied to the simultanious quantification of urinary *S*-phenylmercapturic acid (*S*-PMA) and *trans,trans* muconic acid (*t,t*-MA). The validation of these biomarkers of exposure was now conducted for 186 urine samples from 30 men and women whose personal benzene exposure ranged from nonexposed to 122 ppm over 8 hrs. In these samples we quantified from ND to 3.7 mg *S*-PMA/g creatinine; and from 0.01–52.8 mg *t,t*-MA/g creatinine; with a correlation between these two markers of $r=0.87$ and $p<0.00001$; and *t,t*-MA to *S*-PMA ratio from 6 to 600. In an extention of this study, we developed an HPLC-MS assay for simultaneous quantitation of 1,2,4-trihydroxybenzene (BT), *p*-hydroquinone (HQ) and catechol (CAT). This involved spiking urine sample with [^{13}C]CAT, acid hydrolysis and clean-up, and HPLC-ESI-MS-SIM analysis in the negative ionization mode. Detection limits of ring-hydroxylated metabolites were <0.01 mg/L and coefficient of variation for BT, CAT, and HQ were 8, 14, and 18% respectively. The efficacy and practicality of this assay was evaluated in 15 light cigarette smokers and 12 nonsmokers. Mean±SE levels of BT, HQ, and CAT in smokers' urine were 7.2±1.5, 1.1±0.2, and 3.1±0.6 mg/g creatinine and were not significantly higher than in nonsmokers' urine. **Acknowledgment:** supported by the Health Effects Institute.

#2279 Two dimensional electrophoretic protein profile associated with EMF inhibition of cytostatic effect of tamoxifen on MCF-7 cell growth. Kanitz, M.H., Afzal, S.M.J., Harland, J., Liburdy, R.P., and Savage, R.E. *National Institute for Occupational Safety and Health, Cincinnati, OH 45226, Lawrence Berkeley National Laboratory, Berkeley, CA 94270.*

Epidemiological studies suggest an increased risk of breast cancer is associated with exposure to electromagnetic fields (EMFs). Previous investigations have shown that a 12 mG, 60 Hz magnetic field significantly inhibits the cytostatic action of pharmacological doses of tamoxifen (10^{-7} M) on the growth of MCF-7 human breast cancer cells. Here we use high resolution two dimensional polyacrylamide gel electrophoresis (2D PAGE) to investigate phenotypic biochemical alterations which may represent protein biomarkers of effect of exposure of drug-treated human breast cancer cells to an environmental-level magnetic field. Solubilized MCF-7 cell fractions (20 uG) from each treatment group (control - 2 mG field; control - 12 mG field; tamoxifen - 2 mG field; tamoxifen - 12 mG field) are subjected to 2D PAGE using the Anderson ISODALT method. Computer analysis and comparison of gel patterns across treatment groups were performed using PDQUEST software (BioRad Labs, CA). The protein alterations associated with the observed 12 mG blocking effect of tamoxifen inhibition are characterized with respect to Mr and pI. These studies may provide an understanding at the biochemical level of the EMF-induced reversal of tamoxifen's cell growth inhibition of human breast cancer cells and offer some insight into the putative role of EMF in human breast cancer risk.

#2280 Expression of the MN antigen as a biomarker of lung carcinoma and associated precancerous conditions. Vermylen P, Roufosse C, Ninane V, Sculier JP and Burny A. *Institute Jules Bordet, Hospital Saint-Pierre, B-1000, Brussels, Belgium*

MN protein is expressed by Hela-Fibroblasts fusion hybrids and has been shown to be specifically overexpressed in carcinoma of the cervix, esophagus and kidney. We studied the immunoreactive MN antigen (iMN) expression in non small cell lung carcinoma (NSCLC) and surrounding bronchial epithelium. 42 formalin fixed and paraffin embedded samples were analyzed (Squamous (Sq) : 20, Adenocarcinoma (Adc): 16, Bronchioloalveolar (Bac): 4 and undifferentiated NSCLC : 2). The specimens were submitted to microwave antigen retrieval, primary (M75) monoclonal mouse anti-MN antibody was diluted at 1/5000, secondary biotinylated antibodies, avidin-peroxydase complex and diaminobenzidine technique was used for detection. On the overall, cytoplasmic overexpression of the MN protein was detected in 34/42 (81%) tumor samples (Sq : 18/20, Adc : 12/16, Bac 2/4, undifferentiated 2/2). The iMN distribution was predominantly focal and strong (16/34, 47%) or diffuse and strong (10/34, 30%). No expression was detected in the normal alveolar epithelium, while areas of the bronchial epithelium surrounding the tumors were positive in 10/16 patients. Western blot analysis, in available frozen samples of tumor and matched normal lung, confirmed the immunohistochemical findings. These results suggest that MN protein expression may be a valuable biomarker in NSCLC and associated premalignancy and warrant further studies of this marker in lung cancer. [Supported by FNRS-Télévie 9.4587.95 ; 7.4523.96 and Vesale Foundation]

#2281 Cytokine profiles in human UVB-S and UVB-R irradiated skin. Scordi, I.A., Vincek, V., Taylor, J.R., and Golomb, C. *University of Miami School of Medicine, Miami, FL 33136.*

It has been well documented in recent years that prolonged and/or intense sun exposure is the most important environmental factor contributing to the induction of skin cancer. Skin cancer is the most prevalent human malignancy in the United States with 1,000,000 new cases every year. UVB radiation directly causes mutations in the DNA that lead to cancer. It also causes damage to the immune system. The mechanism(s) by which this immunosuppression in skin is brought about are numerous and complex. Humans can be categorized in two major groups depending on whether they mount a contact hypersensitivity reaction (CH) to an epicutaneously applied hapten following irradiation by UVB (UVB-R) or fail to react (UVB-S). In patients with biopsy proven basal/squamous cell carcinomas, the frequency of the UVB-S trait exceeds 90%. Key players in this carcinogenic cascade are cytokines produced by epidermal cells which are thought to divert the host's response to a more suppressed state allowing the tumor growth and progression. We investigated the production of a number of cytokines in UVB-S and UVB-R individuals following irradiation by RNase protection and have detected the presence of IL-4, 5, 6, 9, 10, 13, as well as the not previously reported IL-14. We have further investigated expression by semi-quantitative RT-PCR and report here different expression patterns in UVB-S and UVB-R skin.

#2282 Molecular cytogenetic screening of epithelial breast cells in nipple aspirate fluid by comparative genomic hybridization. [1]Haddad B, [2]McCormack S, [1]Young H, [2]Trock B, [2]Lippman M, and [2]Dickson B. *[1]Institute for Molecular and Human Genetics and [2]Lombardi Cancer Center, Georgetown University Medical Center, Washington, DC.*

PROCEEDINGS

EIGHTY-NINTH ANNUAL MEETING

American Association for Cancer Research

In Cooperation with the Center for Continuing Education at Tulane University Medical Center

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