adult mice, similar levels of N7-Gly-Gua (~600 adducts per 10^8 nucleotides) and N3-Gly-Ade (~30 adducts per 10^8 nucleotides) were detected in both liver and kidney DNA upon administration of either acrylamide or glycidamide. These data indicate that glycidamide is a reactive intermediate in the activation of acrylamide to a genotoxin.

#5321 Identification of cytochrome P450 enzymes involved in α-hydroxylation of tamoxifen. Sung Yeon Kim, Naomi Suzuki, Y. R. Santosh Laxmi, and Shinya Sibutani. *SUNY at Stony Brook, Stony Brook, NY.*

Increased evidence of developing endometrial and breast cancers are observed in breast cancer patients treated with tamoxifen (TAM) and in healthy women at high risk for breast cancer undergoing TAM chemoprevention. TAM-DNA adducts were detected in the endometrium of women taking TAM (Carcinogenesis 21, 1461-1467, 2000). TAM-DNA adducts are formed primarily through O-sulfonation of α-hydroxytamoxifen (α-OHTAM) and α-hydroxy-N-desmethyltamoxifen (α-OH-N-desTAM). However, it has not been extensively examined which enzyme(s) is involved in α-hydroxylation of TAM. In this study, TAM was incubated in a NADPH regenerating system employing human cytochrome P450 enzymes (CYP 1A1, 1A2, 1B1, 2A6, 2B6, 2C8, 2C9, 2D6, 2E1, 3A4, 3A5, 3A7) and rat enzymes (CYP 3A1 and 3A2). TAM metabolites were analyzed using HPLC/UV and authentic standards (α-OH-N-desTAM, α-OHTAM, 4-hydroxytamoxifen, and N-desmethyltamoxifen). Among these CYP enzymes, CYP 2D6, 3A4 and 3A2 promoted an expected product that has a similar retention time as that of α-OHTAM. The molecular weight was determined using positive ion FAB mass spectroscopy. The parent ion of a product from CYP 2D6 exhibited at m/z 374, confirming that the molecular weight of 4-OH-N-desmethyltamoxifen (4-OH-N-desTAM) as 373 Da. The product from CYP 3A2 exhibited at m/z 388, showed the molecular weight of α-OHTAM as 387 Da. The product from CYP 3A4 was a mixture of α-OHTAM and 4-OH-N-desTAM. Our results highlight on the fact that rat CYP 3A2 and human 3A4 are involved in α-hydroxylation of TAM. (Supported by NIEHS Grant ES 09418.)

#5322 The carcinogen 3-methylcholanthrene elicits sustained upregulation of cytochrome P4501A1 gene expression in rat hepatoma (H4IIE) cells. Sudha Rani Kondraganti, Kathirvel Muthiah, Ling Yu, Weiwu Jiang, and Bhagavatula Moorthy. *Baylor College of Medicine, Houston, TX.*

The cytochromes P450 (CYP) of the 1A family play important roles in the bioactivation of carcinogens to genotoxic metabolites. In this study, we investigated the mechanisms of persistent induction of CYP1A1 by 3-methylcholanthrene (MC) in H4IIE cells, and tested the hypothesis that mechanisms other than persistence of the parent MC contribute to this phenomenon. H4IIE cells were plated overnight at a density of 1×10^6 - 1×10^7 cells/dish in 20 cm culture dishes. Cells were exposed to MC (1 μM) dissolved in dimethyl sulfoxide (DMSO) or DMSO alone for 4-96 h and ethoxyresorufin O-deethylase (EROD) (CYP1A1) activities were determined. The role of CYP1A1 promoter in sustained CYP1A1 induction was studied by transient transfection of cells with a pGL3 luciferase reporter vector containing a 1640 bp CYP1A1 promoter, followed by exposure to MC and determination of luciferase activities at selected time points. MC-exposed cells displayed marked induction of CYP1A1 activities, being maximally induced (20-fold) at 24 h. At 96 h, the induction (≈3-fold) was still maintained. The augmentation of CYP1A1 activities was preceded by induction of CYP1A1 mRNA expression. Interaction of the MC-Ah receptor (AHR) complex with *Ah* responsive elements (AHREs) on the CYP1A1 promoter, as determined by electrophoretic mobility shift assays (EMSAs), indicated that persistent CYP1A1 induction was paralleled by sustained interaction of the MC-AHR complex with the AHREs. Similar to CYP1A1 activities, luciferase activities were persistently induced, with the maximum induction (5-fold) being at 24 h, and persisting until 72 h, suggesting that sustained upregulation of CYP1A1 gene contributes significantly to the persistent CYP1A1 enzyme expression. Experiments with [^{3}H]MC showed that only 0.1% of the parent MC (1 nM) was present in the cells 48 h after MC treatment. Exposure of cells to 1 nM MC did not induce CYP1A1, suggesting that sustained induction of CYP1A1 was mediated by mechanisms other than persistence of parent MC. Further investigations in this cell line model could lead to determination of the molecular mechanisms of long-term CYP1A1 induction by MC *in vivo* , and the possible relevance of this phenomenon to human carcinogenesis. (Supported in part by a NIEHS grant 09132 to BM.)

#5323 Role(s) of cytochrome P4501A2 in the metabolism and bioactivation of 3-methylcholanthrene to genotoxic metabolites *in vitro*. Bhagavatula Moorthy, Kathirvel Muthiah, Sudha Kondraganti, and Weiwu Jiang. *Baylor College of Medicine, Houston, TX.*

Cytochrome P4501A (CYP1A) enzymes are understood to play important roles in the bioactivation of carcinogens such as 3-methylcholanthrene (MC) to genotoxic metabolites. Using the CYP1A2-null mouse model, we reported that hepatic CYP1A2 plays an important role in the sustained induction of CYP1A1 by MC (Kondraganti et al., *J. Pharmacol. Exp. Ther*, 2002, in press). In this study, we tested the hypothesis that CYP1A2, a liver-specific enzyme, plays an important role in the metabolism of MC to metabolites that may play a role in the genotoxicity of MC. Eight week-old female wild-type (C57BL/6J) (WT) mice or CYP1A2-null (KO) mice were treated with MC (100 μmol/kg) or vehicle (corn oil), once daily for 4 days, and liver microsomes were isolated from these animals one day after MC withdrawal. The microsomes (2 mg protein) from WT or KO animals were incubated with [^{3}H]MC (10 μM) (500,000 dpm; Specific activity 10 μCi/mmol) in the presence of NADPH. The concentration of parent MC remaining was estimated by extraction of the incubation mixture with chloroform/methanol, followed by thin-layer chromatography, and determination of radioactivity. The amount of parent MC remaining was about 2-fold higher when microsomes from KO mice were used, compared to WT microsomes, suggesting that MC is a substrate for CYP1A2 in the WT animals. To determine whether MC metabolism could lead to the formation of DNA-reactive metabolites, calf thymus DNA or plasmid DNA containing CYP1A1 promoter (pGL3-1A1) (50 μg) were included in the microsomal incubations, followed by re-precipitation of DNA, and measurement of radioactivity. The amount of radioactivity covalently bound to calf thymus DNA was similar regardless of whether WT or KO microsomes were used, suggesting that CYP1A2 did not play a major role in the bioactivation of MC to DNA-binding metabolites. On the other hand, when pGL3-1A1 plasmid was used, WT microsomes gave rise to significantly higher DNA binding (3,450 ± 250 dpm/mg DNA) than when KO microsomes (1,980 ± 220 dpm/mg DNA) were used. These results support the hypothesis that CYP1A2 bioactivates MC to metabolite(s) that may specifically bind to CYP1A1 gene promoter sequences, a phenomenon that could contribute to the sustained induction of CYP1A1 by MC. (Supported by NIH grant ES09132.)

#5324 Differential induction of CYP1A1 and CYP1B1 in normal human mammary cells exposed to benzo[a]pyrene. Channa Keshava, Diana L. Whipkey, and Ainsley Weston. *Toxicology and Molecular Biology Branch, NIOSH-CDC, Morgantown, WV.*

Induction of CYP1A1 and CYP1B1 could potentially have a direct role (through carcinogen exposure and activation) and an indirect role (through perturbation of steroid metabolism) in human breast carcinogenesis. A panel of 22 primary normal human mammary epithelial cell (NHMEC) strains were developed from tissues discarded at reduction mammoplasty. All women were healthy and tissues were obtained through the Cooperative Human Tissue Network (sponsored by NCI/NDRI). Each cell strain was treated with benzo[a]pyrene (BP; 4uM) for 12 hours. Transcription was monitored using high density oligonucleotide arrays (Affymetrix, HuGeneFL). Total RNA was used for the preparation of labeled targets that were hybridized to microarrays containing probes representing more than 6800 human genes and expressed sequence tags. In all cell strains CYP1A1 [X02612, Cytochrome P(1)-450] transcripts were below the limit of detection before treatment. Similarly, CYP1B1 (U03688) transcripts were also below the limit of detection in 8 out of 22 cell strains before treatment. For 14 of the 22 cell strains CYP1A1 mRNA levels were clearly induced by BP, however, 8 cell strains had basal or reduced levels of CYP1A1 expression following BP exposure. Microarray data for CYP1B1 showed an increase in expression in all cell strains following BP exposure. Nine out of 22 cell strains had at least 5 fold increase in CYP1B1 transcripts and 18 of them had at least 2 fold increase. Microarray expression data for CYP1B1 have been confirmed using quantitative real-time PCR. Since CYP1B1 is thought to be transcriptionally activated by polycyclic aromatic hydrocarbons via the Ah receptor complex, expression of Ah receptor and the aryl hydrocarbon receptor nuclear translocator (ARNT) was analyzed. Altered transcription of these receptors was not observed. These studies provide a complimentary approach to molecular epidemiology for the investigation of differential susceptibility to chemical carcinogens, and specifically polycyclic aromatic hydrocarbons.

#5325 Quantification of benzo[*a*]pyrene-DNA adducts using on-line sample preparation and HPLC with electrospray tandem mass spectrometry. Daniel R. Doerge, Mona I. Churchwell, Linda S. Von Tungeln, Peter P. Fu, Sandra J. Culp, Bernadette Schoket, Erika Gyorffy, Miriam C. Poirier, and Frederick A. Beland. *National Center for Toxicological Research, Jefferson, AR, National Institute of Environmental Health, Budapest, Hungary, and National Cancer Institute, Bethesda, MD.*

Benzo[*a*]pyrene (BaP), a tumorigenic polycyclic aromatic hydrocarbon (PAH), is metabolically activated to 7β,8α-dihydroxy-9α,10α-epoxy-7,8,9,10-tetrahydro-

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