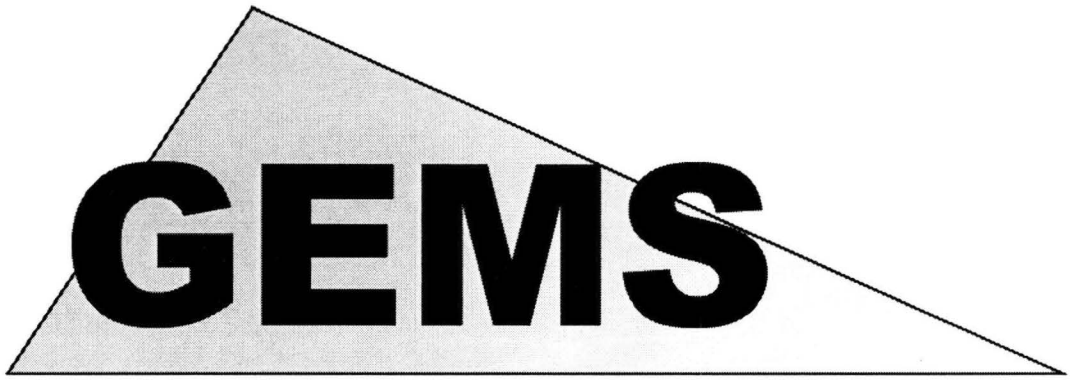


Genetics and Environmental Mutagenesis Society

22nd Annual Fall Meeting

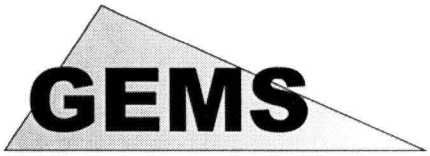
"DNA Methylation and its Toxicological Consequences"

Wednesday, November 10th, 2004

UNC Friday Center
Chapel Hill, NC

GEMS
2004 Board of Directors

* replacing Barbara Shane for remainder of term in 2004.



ANNUAL FALL MEETING

November 10th, 2004

Friday Center of the University of North Carolina
Off of Highway 54 East, Chapel Hill, NC

"DNA Methylation and its Toxicological Consequences"

8:00 - 8:30	Registration and Continental Breakfast
8:30 - 8:45	Welcome: Dr. Mark Miller, GEMS President
	Speaker Introduction: Dr. Barbara Shane, GEMS President-Elect
8:45 - 9:30	Dr. Jay Goodman, Michigan State University *Altered DNA Methylation: An Epigenetic, Secondary Mechanism Involved in Carcinogenesis*
9:30 - 10:15	Dr. Paul Wade, NIEHS *Chromosomal regulation by the methyl CpG binding protein MeCP2*
10:15 -11:45	Exhibit and Poster Viewing/Refreshments
11:45 -12:30	Two or three presentations by students/postdoctoral fellows *To Be Announced*
12:30 - 1:30	**LUNCH**
1:30 - 2:00	Business Meeting
2:00 -2:45	Two or three presentations by students/postdoctoral fellows *To Be Announced*
2:45 - 3:30	Dr. Randy Jirtle, Duke University School of Medicine *Imprinted Genes and Transposons: Epigenomic Targets Linking Prenatal Nutrition to Adult Chronic Diseases*
3:30 - 3:45	Announcement of winners for the poster and presentation Competitions
3:45 - 4:15	***Reception***

Dear GEMS Members,

Welcome to our 22nd annual Fall Meeting. The key person responsible for organizing the meetings is the President-elect, and our current President-elect, Barbara Shane, has done a great job in putting together an outstanding group of speakers on a very interesting and timely topic, *DNA Methylation and its Toxicological Consequences*. Barbara has provided tremendous energy and drive to GEMS, and I can't think of a better individual to take over as president in the coming year.

I would like to extend a warm thank you and appreciation to our corporate sponsors. We could not host these meetings without the very generous financial assistance of our corporate sponsors, many of whom have been long time supporters of our organization. Our sponsors will have tables set up to exhibit their most up-to date products and laboratory equipment. Please be sure to stop by and talk with them about your laboratory needs and products of interest, and thank them for their continuing support.

Organizing the Fall meeting requires the help and dedication of a small and dedicated cadre of volunteers. One of the great things about GEMS is the great people you get to work with, and I would encourage all of you to get involved and volunteer. Having served on the Board as a Councilor for 3 years and then as President-elect and President for 2, I can say that I have very much enjoyed working with this great group of people. Susan Ross has done an outstanding job as our Treasurer, and we should acknowledge the support of CIIT Centers for Health Research which has hosted our bimonthly Board meetings and supplied the meeting badges and registration materials. A special thanks to our secretary, Gloria Jahnke, and our Newsletter Editor, Stephen Little, who provide a tremendous amount of logistical support for GEMS. I also want to express my appreciation to our current Board members, Stephen Little, Mary Smith, Tasha Smith, Greg Stuart, and Alan Townsend, as well as our outgoing Councilors Jane Allen, Jennifer Hu, Scott Jenkins, and Velva Milholland. Jane Allen has done a wonderful job putting together the student career workshops for the past Spring meetings, an effort which I am sure will continue.

There are a number of other people who deserve special recognition for their constant efforts on behalf of GEMS. These include Carolyn Harris, who has maintained our membership list for several years, Frank Stack, our dedicated Webmaster, Kristine Witt, who every year takes the time and effort to arrange for the award plaques and coffee mugs for both the Fall and Spring meetings, and Ed Krehl, who has taken over as our Corporate Sponsor Representative.

One of the most important things we do at GEMS is to encourage the growth and development of our young scientists. The GEMS meeting is a great time to learn some science and schmooze with your colleagues, and I would encourage our junior colleagues to take full advantage of this opportunity. As usual, we will have the Best Student Talk and the Best Poster awards. This year, we have divided the awards into 3 categories: student, postdoctoral, and technician, to acknowledge their separate but important contributions to our society. The winner of the Best Student Talk serves on the Board as the Student Representative. Mary Kushman from Wake Forest University served in that capacity this past year, and we appreciate her input at our meetings.

I would also like to acknowledge the generous financial support of Dr. Kenneth Olden, Director of the National Institute of Environmental Health Sciences (NIEHS), who has continually been a strong supporter of GEMS and its scientific programs.

I have very much enjoyed my 5 year tenure as a member of the GEMS Board. It has been an honor and privilege to serve as your President

Mark S. Miller
GEMS President

A special thanks to NIEHS and CIIT Centers for Health Research for their financial support

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Invited Speaker Presentations

S1 ALTERED DNA METHYLATION: AN EPIGENETIC, SECONDARY MECHANISM INVOLVED IN CARCINOGENESIS

Jay I. Goodman
Michigan State University, Department of Pharmacology and Toxicology

This presentation is addressed towards reconciling the well established role for mutagenesis in carcinogenesis with the fact that not all carcinogens are mutagens and the view that nonmutagenic events also underlie carcinogenesis. This apparent paradox may, in part, be resolved through a consideration of the roles that altered DNA methylation can play in the transformation of a normal cell into a frank malignancy.

Inheritance should be considered on a dual level. The transmission of genes from generation to generation (i.e., inheritance of DNA base sequence) is distinct from the mechanisms involved in the transmission of alternative states of gene activity following cell division. Epigenetics is the term used to describe the latter. DNA methylation (the presence of 5-methylcytosine (5MeC) as compared to cytosine) is one epigenetic mechanism by which gene activity may be regulated. Altered DNA methylation may facilitate the aberrant gene expression underlying carcinogenesis. The basic hypothesis explored in my laboratory is that susceptibility to carcinogenesis is related inversely to the capacity to maintain normal patterns of DNA methylation

The secondary mechanism concept, "why bother?" In the broad sense, carcinogens may be classified as acting through a genotoxic or nongenotoxic mode of action. A genotoxic mode is operative in those situations where the chemical or a metabolite interacts with DNA directly. Nongenotoxic carcinogens act through a mode of action that does not involve direct DNA damage. The nongenotoxic compounds can be viewed as acting through a secondary mechanism of carcinogenesis. This term implies that the carcinogen produces a tumor response through the obligate action of a secondary factor, e.g., gastrin-mediated stomach neoplasia produced with long-acting histamine H2 antagonists; target organ toxicity, as in bladder tumors secondary to urinary tract calculi; and physiologic disturbances, such as endocrine tumors demonstrated to be secondary to chemically induced hormone imbalance. Thus, the secondary factor is responsible for the tumor response independent of the chemical per se. The cancer process is linked to the involvement of a key step(s) involving a biochemical/molecular alteration produced at some threshold of exposure. In a hypothesis-driven fashion, altered DNA methylation may be viewed as a secondary mechanism. In addition to the theoretical implications regarding mechanisms underlying carcinogenesis, the practical implication is that data demonstrating that a chemical is acting by a secondary mechanism can be used to establish a "safe" exposure level. The some-risk-at-all-dose assumption typically made for carcinogens is not required when the induction of tumors is a consequence of a secondary mechanism operative only at some threshold of exposure.

S2 CHROMOSOMAL REGULATION BY THE METHYL CPG BINDING PROTEIN MECP2

Paul A. Wade
NIEHS, Research Triangle Park, NC 27709

MeCP2 is a transcriptional repressor that contains an N-terminal methylated DNA-binding domain, a central transcription regulation domain, and a C-terminal domain of unknown function. Whereas current models of MeCP2 function evoke localized recruitment of histone deacetylases to specific methylated regions of the genome, it is unclear whether MeCP2 requires DNA methylation to bind to chromatin or whether MeCP2 binding influences chromatin structure in the absence of other proteins. To address these issues, we have characterized the complexes formed between MeCP2 and biochemically defined nucleosomal arrays. At molar ratios near 1 MeCP2/nucleosome, unmethylated nucleosomal arrays formed both extensively condensed ellipsoidal particles and oligomeric suprastructures. Furthermore, MeCP2- mediated chromatin compaction occurred in the absence of monovalent or divalent cations, in distinct contrast to all other known chromatin-condensing proteins. Analysis of specific missense and nonsense MeCP2 mutants indicated that the ability to condense chromatin resides in region(s) of the protein other than the methylated DNA-binding domain. These data demonstrate that MeCP2 assembles novel secondary chromatin structures independent of DNA modification and suggest that the ability of MeCP2 to silence chromatin may be related inpart to its effects on large-scale chromatin organization.

S3 IMPRINTED GENES AND TRANSPOSONS: EPIGENOMIC TARGETS LINKING PRENATAL NUTRITION WITH ADULT DISEASE SUSCEPTIBILITY

Randy L. Jirtle, Ph.D.
Duke University Medical Center, Durham, NC 27710 USA

Human epidemiologic and animal data indicate that susceptibility to adult-onset chronic diseases such as cardiovascular disease, diabetes, obesity, and cancer is influenced by persistent adaptations to prenatal and early postnatal nutrition (Waterland and Jirtle, Nutrition 20: 63-68, 2004). Two potential epigenomic targets for early nutritional effects are imprinted genes and transposons. Imprinting is an epigenetic form of gene regulation that results in monoallelic parent-of-origin dependent gene expression (Murphy and Jirtle, BioEssays 25: 577-588, 2003). IGF2 loss of imprinting is associated with an increased incidence of cancer (Cui et al., Science 299: 1753-1755, 2003), and Beckwith-Weidemann syndrome in children conceived by in vitro fertilization (Niemitz and Feinberg, Am. J. Hum. Genet. 74: 599-609, 2004). We have now demonstrated in mice that early postnatal dietary methyl deficiency, and even exposure to a nutritionally complete synthetic diet, high in fat but low in fiber, causes biallelic expression of the oncogene, Igf2. Using viable yellow agouti (Avy) mice, which harbor a retrotransposon upstream of the agouti gene, we also showed that maternal dietary methyl donor supplementation during pregnancy alters coat color of the offspring via increased CpG methylation at the Avy locus rather than by genetic mutation (Waterland and Jirtle, Mol. Cell. Biol. 23: 5293-5300, 2003). Moreover, this epigenetic change reduces the susceptibility of the offspring to obesity,

diabetes, and cancer - a clear example of "nature via nurture." Our findings provide evidence that epigenetic alterations of transposons and imprinted genes can directly link environmental conditions during early development to the etiology of adult diseases. Further understanding these associations should make possible the development of early-life nutritional interventions or corrective therapies aimed at preventing chronic human diseases. (Supported by the NIH grants CA25951 and ES08823, and the Dannon Institute)

Oral Presentations

***T1** ASSESSING THE ROLE OF CYTOSOLIC PHOSPHOLIPASE A2 (cPLA2) INHIBITION IN PHTHALATE ESTER TOXICITY, Rebecca A. Clewell, Susan M. Ross, Kevin W. Gaido and Melvin E. Andersen, CIIT Centers for Health Research, RTP NC 27709

Exposure to certain dialkylphthalate plasticizers, including dibutylphthalate and diethylhexylphthate, during fetal development of male rats has been shown to cause testicular malformations. These effects are associated with the monoalkylester metabolites (MBP and MEHP) and their inhibition of testosterone (T) synthesis. Currently, risk assessment efforts for these chemicals are limited by uncertainty surrounding their mode of action. In the adult, steroidogenesis requires LH signaling through two pathways: c-AMP induction and arachidonic acid (AA) release by cPLA2. However, molecular targets within these pathways are not known. We propose that monoalkyphthalates like MEHP compete with the AA portion of membrane phospholipids for the Ca+2 binding domain of cPLA2, thereby reducing T synthesis. Dose-response modeling and in vitro steroidogenesis studies were used to test this hypothesis. Using models fit to published data, the concentration of MEHP causing 50% inhibition (IC50) of high affinity cPLA2 activity from human platelets was 10 uM. A similar IC50 (~4 uM) was determined for MEHP inhibition of fetal T production using a kinetic model for in vivo exposure in the female (gestating) rat. We also studied the effect of MEHP, and cPLA2-inhibitor chloroquine (CQ), on LH-stimulated progesterone (PG) synthesis in MA-10 Leydig cells. Assays were run at a maximum stimulating LH concentration (100 ng/mL LH) with inhibitor concentrations of 1, 3, 6, 10, 30, 60 and 100 uM. Both CQ and MEHP appear to inhibit PG. Based on the measured data, the model-predicted IC50 for MEHP inhibition of PG production in MA-10 cells is 15 uM. This value is consistent with cPLA2 inhibition having a role in phthalate toxicity. Additional work using molecular modeling to evaluate inhibitor binding for the Ca+2 domain of cPLA2 and studies assessing inhibition of cPLA2 from various cell types will be important for confirming cPLA2 as a target of these phthalates

***T2** NUCLEOTIDE EXCISION REPAIR ACTIVITY IN BREAST CANCER PATIENTS AND HEALTHY CONTROLS, Isaac V. Snowhite[1], Edward A. Levine[4,5], Rita I. Freimanis[2], L. Joseph Su[6], Eunkyung Chang[1], and Jennifer J. Hu [1,3,5,6], [1]Departments of Cancer Biology, [2]Radiology and [3]Public Health Sciences, [4]Surgery, and [5]Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157, USA. 6Department of Public Health, Louisiana State University Health Science Center, New Orleans, LA 70112

Because many of the DNA adducts generated by some breast cancer-related carcinogens, including polycyclic aromatic hydrocarbons, heterocyclic amines, and pesticides, are removed by the nucleotide excision repair (NER) pathway, we tested the hypothesis that breast cancer is associated with NER activity, measured by a modified plasmid-based host reactivation assay. Using cryopreserved lymphocytes collected in an ongoing, clinic-based case-control study, our results showed that the mean NER activity was significantly higher (P = 0<0.0001) in 301 cases (mean +/- SD, 4.57 +/- 2.75) than in 359 controls (3.90 +/- 2.16). There was a significant association between above-median NERC and breast cancer risk: odds ratio (OR), 1.45; 95% confidence interval (CI), 1.06-1.98, after adjustment for age and age at first live birth. This association was stronger in pre-menopausal subjects (OR, 1.65; 95% CI, 1.15-2.38) compared with post-menopausal subjects (OR, 1.01; 95% CI, 0.56-1.81). When we stratified NER values by quartiles of the total study population, there was a significant dose-dependent association between higher NER and elevated breast cancer risk (p=0.004, test for linear trend. Compared with the lowest quartile of NER as the referent group, the adjusted ORs for the 25th, 50th, and 75th quartiles were: 0.82 (95% CI, 0.53-1.28); 1.02 (95% CI, 0.66-1.58); and 1.71 (95% CI, 1.01-2.66), respectively. In contrast to our previous findings, the results from this study suggest that higher NER is associated with breast cancer risk in pre-menopausal women. The implications are: (i) other repair pathways or genetic defects may play more important roles; (ii) NER fidelity may need to be evaluated in addition to kinetics.

***T3** THE E2F/RB PATHWAY REGULATION OF DNA REPLICATION AND PROTEIN BIOSYNTHESIS, W. O. Ward[1], Harry Hurd[2]and R. J. Duronio[2], [1]EPA, MD B143-06, RTP, NC 27711 and [2]UNC, Chapel Hill, NC 27599

The E2F/Rb pathway plays a pivotal role in the control of cell cycle progression and regulates the expression of genes required for G1/S transition. Our study examines the genomic response in Drosophila embryos after overexpression and mutation of E2F/Rb pathway molecules. Hierarchical clustering was used to analyze the genomic expression pattern and select genes co-expressed with established E2F target genes. These experiments have identified many genes involved in chromosome duplication, as well as novel E2F-regulated genes that are not expressed coordinately with the cell cycle during embryogenesis. A selected set of novel E2F-regulated genes were analyzed with in-situ hybridization. Some displayed expression patterns typical of E2F targets and some displayed novel patterns. The regulatory region of genes co-expressed with known E2F targets were analyzed for presence of E2F binding sites. We also demonstrate that E2f1 and Rbf1 regulate the expression of ribosomal protein genes. Mutation of E2f1 or Dp and overexpression of Rbf1 increase and reduce ribosomal protein (RP) expression, respectively. This suggests that E2f1/Dp/Rbf1 act through a common molecular mechanism, perhaps as part of a repressor complex. This complex may not act directly on RP genes since inspection of their regulatory regions did not show an enrichment of E2f binding sites. In addition, E2f1 and Rbf1 also regulate the expression of Rack1, a modulator of the insulin receptor growth pathway. Thus the E2f/Rb pathway influences the expression of genes that implement and regulate cell growth. [This abstract does not necessarily reflect EPA policy.]

***T4** ATM-DEPENDENT GENE EXPRESSION CHANGES IN RESPONSE TO EXPOSURE TO IONIZING RADIATION, Cynthia L. Innes[1], Tong Zhou[2], Jianying Li[3], Pierre

R. Bushel[3], William K. Kaufmann[2] and Richard S. Paules[1,3], [1]Growth Control and Cancer Group, NIEHS, Research Triangle Park, NC 27709, [2]Dept. of Pathology and Lab. Medicine, UNC School of Medicine, Chapel Hill, NC 27599 and [3]NIEHS Microarray Group, NIEHS, Research Triangle Park, NC 27709

Ataxia telangiectasia (AT) is an autosomal recessive disorder characterized by neuronal degeneration, telangiectasias, acute cancer predisposition, and hypersensitivity to ionizing radiation (IR). The gene defective in AT, ATM (for AT-mutated), encodes a protein, pATM that has been found to have IR-inducible kinase activity. Cells from individuals with AT exhibit severely attenuated cell cycle checkpoints in response to gamma radiation exposure. pATM has been hypothesized to act as part of a complex that senses DNA damage, in particular, DNA double strand breaks. We are studying the pATM-dependent gene expression responses to a dose of 1.5 Gy radiation in lymphoblastoid cell lines from multiple individuals with either wild type or mutated ATM. The gene expression analyses were performed on Agilent Human 1A Oligo chips containing approximately 16,000 60mer probes. We identified a set of genes whose gene expression changes are ATM-dependent following exposure to 1.5 Gy IR. This set of genes was tested by real time quantitative PCR analysis in order to obtain a signature set of genes that could potentially be used clinically to aid in optimizing therapeutic strategies for cancer patient care.

***T5** ASSOCIATION BETWEEN POLYMORPHISMS IN PHASE I AND PHASE II ENZYMES AND MUTATIONS IN *P53*, Beth O. Van Emburgh[1], Jennifer J. Hu[1,2], Edward A. Levine[3], Libyadda J. Mosely[1], Sommer N. Knight[1], Nancy D. Perrier[3], Jessica Watkins[1], Elisabeth Curtis[1], Gary B. Sherrill[4], Mark C. Willingham[5], and Mark S. Miller[1], Departments of Cancer Biology[1], Public Health Science[2], Surgery[3], and Pathology[5], Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27157, Moses Cone Health System[4], Greensboro, NC 27403.

Breast cancer is the second leading cause of cancer related deaths among women in the United States. As both environmental and endogenous agents have been implicated in the etiology of breast cancer, many studies have focused on the relationship between metabolism of these compounds and breast cancer. Standard case/control studies have suggested that polymorphisms in metabolic enzymes that result in increased activation or decreased detoxification of carcinogens or estrogens may be potential risk factors for breast cancer. However, few attempts have been made to compare the ability of breast tissue to metabolize chemical carcinogens with the types of mutations observed at critical oncogenic loci. In this pilot study, 112 Caucasian breast cancer cases were analyzed for association between polymorphisms in metabolic enzymes, *GSTP1* and *CYP1B1,* and *p53* mutations in their tumor tissue. The *CYP1B1* A119S and *GSTP1* I105V polymorphisms have been shown to alter the metabolic properties of the enzymes by either increasing activation or decreasing detoxification of substrates respectively. Although not significant, the odds ratio [OR] for the IV and VV genotypes of *GSTP1* were 1.88 and 3.0, respectively (95% confidence interval [95%CI] = 0.49-7.42 and 0.45-19.46, respectively). The OR for the AS and SS genotypes of *CYP1B1* were 1.7 and 4.25, respectively (95%CI= 0.46-6.35 and 0.43-38.35, respectively). These results suggest that these polymorphisms have a potential impact on the risk of having a mutation at *p53*. With

limited sample size, these preliminary results support our hypothesis that women with polymorphisms in genes important in carcinogen and estrogen metabolism are more likely to have mutations in tumor suppressor genes.

Poster Presentations

P1 SECONDARY GENETIC ALTERATIONS IN LUNG TUMORS POSSESSING A PRIMARY Ki-*ras* MUTATON, Heather S. Floyd, Jamie Jennings-Gee, Nancy D. Kock, Mark S. Miller, Wake Forest University School of Medicine, Winston-Salem, NC

Mutation of the Ki-*ras* gene is an early event in spontaneous and chemically-induced murine lung tumors. We previously described a mouse lung cancer model whereby the mutanthuman Ki-ras^{G12C}allele is expressed specifically in the lung in a doxycycline (DOX)-inducible manner. Mice treated with DOX developed hyperplastic lesions as early as 12 days and adenomas were first visible by 3 months. Adenomas were present up to 12 months with relatively no tumor progression. To identify secondary genetic alterations, lung adenomas from transgenic mice induced by 9 and 12 months of DOX treatment were analyzed for alterations in expression of $p16^{Ink4a}$, *retinoblastoma* (*Rb*), and cyclin D1 by real-time PCR. Adenomas isolated from mice treated for 9 or 12 months with DOX exhibited decreased expression of $p16^{Ink4a}$ in 7/22 (32%) and 1/6 (17%) of the adenomas, respectively, and displayed reduced expression of *Rb* in 21/22 (95%) and 6/6 (100%) of the adenomas, respectively. No alterations in expression of cyclin D1 were observed. Analysis of lung adenomas for mutations in $p16^{Ink4a}$ by SSCP demonstrated that all the tumors (33) analyzed thus far exhibited the wild type sequence. The Ki-ras^{G12C} mice display a relatively benign tumor phenotype, exhibiting mostly hyperplasias and adenomas, with few tumors progressing to carcinomas. These data suggest that alterations in $p16^{Ink4a}$ and *Rb* play an important role in the early stages of lung tumorigenesis. (Supported by NCI grant CA91909).

***P2** PHOSPHORYLATION AT THR18 IN HISTONE H1.4 BY CYCLIN DEPENDENT KINASES MODULATES GLUCOCORTICOID RECEPTOR-MEDIATED GENE ACTIVATION, Maureen K. Bunger, Leesa J. Deterding, Kenneth B. Tomer, and Trevor K. Archer, [1]Chromatin and Gene Expression Group, Laboratory of Molecular Carcinogenesis, and [2]Laboratory of Structural Biology. NIEHS, NIH, DHHS, Research Triangle Park, NC 27709.

Glucocorticoids are important endocrine modulators of metabolism and immunity. Synthetic glucocorticoids, such as dexamethasone (dex) and prednisone, are used clinically for long-term treatments of many autoimmune diseases and are critical tools in transplantation medicine. To study the mechanistic effects of long-term glucocorticoid treatment on gene transcription, we have utilized the mouse mammary tumor virus (MMTV) promoter that is regulated by the DNA binding glucocorticoid receptor (GR). We have previously observed that long-term dex treatment renders the MMTV promoter refractory to further stimuli. We found that prolonged GR activation of MMTV alters the MMTV promoter structure, within the chromatin context, such that it becomes resistant to re-activation by further dex treatment. One such alteration of

the chromatin that occurs during long-term exposures is the loss of phosphorylated histone H1 and subsequent replacement with a non-phosphorylated H1. These results indicated that the presence of a phosphorylated H1 at the MMTV promoter in chromatin is required for activation of MMTV by the GRin vivo. Using mass spectrometry (MS), we show that specific isoforms of H1 and sites of phosphorylation are not equivalent, implying a specific "code" for H1-phosphorylation in the regulation of certain hormone responsive promoters. Using MS, we quantified the loss of phosphorylation following prolonged dex treatment and found only two of these isoforms, H1.3 and H1.4 were significantly dephosphorylated in vivo. To identify the specificsites of phosphorylation, we performed tryptic digests of purified H1 from both human and mouse cell lines followed by LC/MS/MS. These analyses are ongoing, but to date we have identified three active phosphorylation sites in the human and mouse H1.4 isoforms, and two active phosphorylation sites in the mouse H1.3 as consensus target sites for cyclin dependent kinases, cdk1 and cdk2. We have generated a series of mutations in these cdk consensus sites in the mouse H1.3 isoform to analyze in cell culture systems that allow controlled reconstitution of MMTV activation by glucocorticoids. We have found that over-expression of H1.3 in cells strongly represses MMTV activation. Furthermore, mutation of one of the phosphorylation target threonines in H1.3 to a glutamate relieves this repression by 50%. These systems allow us to combine structural and genetic approaches towards de-coding the role of H1 phosphorylation in hormone responsive gene regulation.

P3 THE GENETIC ALTERATIONS IN CANCER DATABASE: PATTERNS OF GENE CHANGES IN CANCERS BASED ON SPECIES, TUMOR SITE, OR TEST AGENT, M.A. Jackson[1], I. Lea1, A. Rashid[2], and J. K. Dunnick[3], [1]ILS, Inc., RTP, NC,27709, [2]AGTI, Raleigh, NC, 27609 and [3]NIEHS, RTP, NC, 27709

The Genetic Alterations in Cancer (GAC) database is a web-based program that catalogs and displays published data from studies of genetic mutations in tumors associated with exposure to chemical, physical, or biological agents. All information is procured from peer reviewed articles published in the open literature. Results from studies in humans, mice, and rats are included and are organized according to species, tumor type and origin, target organ, and agent. Data mining functions display results from user specified queries in graphs and tables for comparative analysis. The percentage of tumors with mutations (incidence) in each gene evaluated is shown. Detailed data tables, mutation spectra, reference lists (including access to PubMed abstracts), study information (e.g., experimental details, exons evaluated, and analytical technique), and links to information from the NCI Cancer Gene Anatomy Project are also provided. Program features and results from the evaluation of lung and liver tumor data are presented and discussed. The overall mutation incidences in genes from DEN-induced mouse compared to rat liver tumors is similar for beta catenin (Catnb), Kras2, and Tp53. Although the mutation incidence in Catnb is similar in both species, the mutation spectra show that AT>GC transitions occur two times more frequently in the mouse. The mean mutation incidence from 18 studies of Hras1 mutations in mouse tumors is 20% compared to 0% from two studies in rats. The Kras2 mutation spectra for spontaneous, urethane-, and NNK-induced tumors in mouse lung each has a unique pattern with AT>GC, AT>TA, and GC>AT, being the primary mutation, respectively. Likewise, the Kras2 mutation spectrum for spontaneous lung tumors in mice (40% AT>GC; 11% GC>TA) differs significantly from that in humans (0% AT>GC); 61% GC>TA). The data in GAC along

with data from other sources (e.g., gene expression and SNPs databases) will aid future investigations of environmental and genetic factors involved in tumor development. Sponsored by the National Institutes of Environmental Health Sciences.

***P4** DIFFERENTIAL PROTECTION AGAINST TOXICITY OF DIBENZO[A,L]PYRENE METABOLITES IN CELLS EXPRESSING CYP1A1 OR CYP1B1 AND HUMAN GSTA1-1, Mary E. Kushman[1], Sandra Leone-Kabler[2], Melissa H. Fleming[2], Charles S. Morrow[1,2], Johannes Doehmer[3], and Alan J. Townsend[1,2], [1]Department of Cancer Biology and Comprehensive Cancer Center, and [2]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem NC 27157; [3]GenPharmTox, Munich, Germany.

Dibenzo[a,l]pyrene (DB[a,l]P) is a fjord-region PAH with exceptional genotoxicity in biological systems. It is metabolized by CYP1A1 and CYP1B1 to reactive diol epoxides capable of binding to cellular macromolecules. The human alpha class GST enzymes are particularly effective in conjugating GSH with reactive DB[a,l]P-diol epoxides, thus rendering the compounds less toxic. However, the quantitative nature of detoxification enzyme expression and effects upon the ability of the enzymes to protect against chemically-induced damage is unknown. To investigate this question, we developed V79MZ hamster lung fibroblasts stably transfected with human cytochrome P450 1A1 (V79MZh1A1) or P4501B1 (V79MZh1B1) alone or in combination with human GST alpha-1 (hGSTA1-1) and used them to examine GST protection against DB[a,l]P and DB[a,l]P-diol (DB[a,l]PD) cytotoxicity and mutagenicity. As predicted, DB[a,l]P and DB[a,l]PD had higher cytotoxicity in V79MZ1A1 (IC50 = 2.7 nM, vs. 6.0 nM in V79MZ1B1 for DB[a.l]P; 0.7 nM vs. 4.8 nM in V79MZ1B1 for DB[a,l]PD). Expression of hGSTA1-1 yielded a 2-fold protection over V79MZ1A1 alone for parent dibenzo[a,l]pyrene, and a slight 1.3-fold protection against the diol. Protection against mutagenicity of these compounds was comparable to that for cytotoxicity. In V79MZh1B1, added expression of hGSTA1-1 conferred up to 5-fold protection against cytotoxicity of DB[a,l]P relative to cells expressing hCYP1B1 alone. GSTA1-1 in cells expressing hCYP1B1 conferred up to 9-fold protection against the (+/-)-DB[a,l]P-dihydrodiol relative to the respective control expressing hCYP1B1 alone. Relative fold- protection conferred by GST expression against cytotoxicity was proportionate to the GST specific activities. Protection against mutagenicity of the DB[a,l]P or its dihydrodiols metabolites at the hprt locus was also observed, but weaker than the protection against cytotoxicity. Fold protection against either the parent DB[a,l]P or the (+/-)-DB[a,l]PD was similar, ranging from 1.3- to 1.8-fold. These findings demonstrate that protective efficacy of GST against DB[a,l]P and DB[a,l]PD toxicity can vary depending upon the route of activation of the compounds, the relative amount of enzyme present, and the specific cellular damage end-point examined.

***P5** GENE EXPRESSION PROFILING FOLLOWING IN UTERO EXPOSURE TO A PANEL OF PHTALATE ESTERS REVEALS NEW GENE TARGEST IN THE ETIOLOGY OF TESTICULAR DYSGENESIS, Kim P. Lehmann[1], Kejun Liu[1, 2], Madhabananda Sar[1], S. Stan Young[2], and Kevin W. Gaido[1], [1]CIIT Centers for Health Research, Research Triangle Park, NC 27709, [2]National Institute for Statistical Sciences, Research Triangle Park, NC 27709

Male reproductive tract abnormalities associated with testicular dysgenesis in humans also occur in male rats exposed gestationally to some phthalate esters. We examined global gene expression in the fetal testis of the rat following in utero exposure to a panel of different phthalates, including several phthalates known to similarly disrupt male rat reproductive tract development. Pregnant Sprague-Dawley rats were treated by gavage (1ml/kg) daily from gestation days 12 through 19 with corn oil vehicle or diethyl (DEP), dimethyl (DMP), dioctyl tere- (DOTP), dibutyl (DBP), diethylhexyl (DEHP), dipentyl (DPP), or benzyl butyl (BBP) phthalate in corn oil at 500 mg/kg/day. Testes were isolated on gestation day 19, and global changes in gene expression were determined. Of the approximately 30,000 genes queried, 391 genes were significantly altered following exposure to the developmentally toxic phthalates (DBP, BBP, DPP, DEHP) relative to the control. No significant changes in gene expression were detected in the non-developmentally toxic phthalate group (DMP, DEP, and DOTP). Gene pathways disrupted include the previously identified cholesterol transport and steroidogenic pathways as well as genes involved in intracellular lipid and cholesterol homeostasis, transcriptional regulation, and response to oxidative stress. By immunohistochemistry we show that Dax1 is specifically reduced in germ cells and CCAAT/enhancer binding protein beta (C/EBPß) is specifically reduced in the interstitial cell compartment. Together these results indicate that developmentally toxic phthalate phthalates are remarkably similar in their effects on gene expression and the gene pathways targeted by these phthalates give new insight into the molecular pathways involved in the development of testicular dysgenesis.

***P6** NOVEL METHODS FOR TARGET PROTEIN IDENTIFICATION USING IMMUNOPRECIPITATION - LC/MS/MS, WM Winnik, RD Grindstaff, L Copeland and MD Ward, US EPA RTP NC 27711

Proteomics provides a powerful approach to screen and analyze responses to environmental exposures which induce alterations in protein expression, phosphorylation, ubiquitinylation, oxidation, and modulation of general proteome function. Post-translational modifications (PTM) of proteins have been shown to be associated with key events in tumorgenesis, altering cell cycle progression, apoptosis induction, cellular differentiation, and DNA repair.

In these studies, we focus on identifying specific subsets of the proteome using immunopurification of targeted proteins. After immunoprecipiation, all proteins were size-separated by SDS-PAGE, then followed by in-gel tryptic digestion. Finally, peptides could be identified by nano-flow LC/MS/MS. By immunoprecipitating proteins that are ubiquitinylated and/or phosphorylated we could focus on post-translational modifications that may be related to a biomarker of chemical exposure and/or disease state. To identify PTM of proteins, we first coupled anti-ubiquitin, anti-phosphotyrosine and anti-phosphothreonine to protein G coated Sepharose beads, then incubated control or chemically treated whole cell lysates with the immune complex.

Also, we are developing a method for the purification and subsequent characterization of IgE inducing proteins in mold extracts (*Metarhizium anisopliae mycelium*) by immunoprecipitating allergen specific immunoglobulins. Briefly, by covalently linking an anti-IgE antibody to chemically modified magnetic beads, we were then able to pull down the IgE from mouse serum

derived from *M. anisopliae* immunized mice. This anti-IgE B IgE complex was used to immunoprecipitate the proteins in mold extracts that induce an IgE immune response. In conclusion, by immunopurifying target proteins from a complex mixture we selected a precise subset of the proteome, resulting in less complex and improved protein identification and analysis. (This abstract does not necessarily reflect EPA policy)

P7 ZIDOVUDINE IS THE MUTAGENIC COMPONENT OF COMBINATION ANTIRETROVIRAL DRUG THERAPY ADMINISTERED TO CD-1 MOUSE PUPS IN A TREATMENT REGIMEN SIMILAR TO THAT USED IN HUMANS FOR PREVENTION OF MOTHER-TO-CHILD TRANSMISSION OF HIV, Kristine L. Witt[1], Raymond R. Tice[2], Gary W. Wolfe[3], Jack B. Bishop[1], [1]National Institute of Environmental Health Sciences, RTP, NC, 27709 [2]Integrated Laboratory Systems, RTP, NC, 27709 [3]Gene Logic, Gaithersburg, MD 20879

We previously reported extraordinary increases in micronucleated erythrocytes in CD-1 mouse pups exposed to 3'-azido-3'-deoxythymidine (Zidovudine; ZDV; AZT) and dideoxyinosine (ddI) (50/250, 75/375, 150/750 mg/kg/day ZDV/ddI) by gavage throughout gestation and lactation, followed by direct pup dosing beginning postnatal day (PND) 4 (Bishop et al., 2004). That study was conducted to explore the potential for induced genetic damage in infants born to HIV-infected women treated with antiretrovirals for prevention of maternal-infant transmission of the virus. The results prompted additional studies to clarify the relative contribution of each drug to the observed genetic damage in erythrocytes. Thus, pregnant CD-1 mice were administered ZDV (50, 75, 100, 150 mg/kg/day), ddI (250, 375, 750 mg/kg/day), or a third commonly used antiretroviral nucleoside lamivudine (3TC) (25, 50, 100 mg/kg/day) by gavage twice daily in equal fractions throughout gestation and lactation. Direct pup dosing (same regimens) began on PND 4. Peripheral blood erythrocytes of male pups were screened for micronuclei on PND 1, 4, 8, and 21. Significant increases in micronucleated erythrocytes were observed in pups and dams exposed to ZDV at all doses and sampling times. The highest frequencies of micronucleated cells were observed in pups on PND 8, after the initiation of direct dosing. In contrast, effects seen with ddI or 3TC were minimal. These results demonstrate that ZDV, a component of many anti-HIV drug combination therapies, induces chromosomal damage in perinatally exposed neonatal mice. Furthermore, comparison of the frequencies of micronucleated cells induced by ZDV alone or in combination with ddI suggests that ddI potentiates ZDV-induced chromosomal damage following direct gavage exposure

***P8** DIFFERENTIAL PROTECTION AGAINST CYTOTOXICITY, GENOTOXICITY AND MUTAGENICITY OF AFLATOXIN B1 IN CELLS EXPRESSING HUMAN P4501A2 AND EITHER HUMAN GST MU1 OR MOUSE GST ALPHA3, Sandra Leone-Kabler[1], Mary E. Kushman[2], Charles S. Morrow[1,2], Johannes Doehmer[3], and Alan J. Townsend[1,2] [1]Department of Biochemistry, [2]Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC, 27157, and [3]GenPharmTox, Munich, Germany.

Aflatoxins are mycotoxins commonly found in nature. Aflatoxin B1 (AFB1) has a wide range of biological activities affecting animals, including acute toxicity, teratogenicity, mutagenicity and

carcinogenicity. It is often found as a contaminant in foodstuffs such as corn, peanuts and other grains. Cytochrome p450s (CYP) are involved in the metabolism of AFB1. Conjugation of AFB-8,9-oxide (AFBO) to glutathione, mediated by glutathione-S-transferases, is regarded as an important detoxification step. The key human GSTM1 is deleted in half the population, but its role is in protection is unknown since it has weaker activity for AFBO conjugation than the most active rodent GSTs. This study investigated the role of hCYP1A2 in activating AFB1 to AFBO, and the subsequent detoxification by conjugation with specific glutathione-S-transferases. To study the interaction of these Phase I and Phase II enzymes, we utilized v79MZ (v79) hamster lung fibroblasts stably transfected with human cytochrome P450 1A2, then super-transfected with either human GST Mu-1 (v79h1A2/hGSTM1, clone 9) or mouse GST alpha-3 (v79h1A2/mGSTA3, clone 42). A cell line transfected with empty vector □pCEP4□(v79h1A2 □10) was also tested as a control. Mice are resistant to the genotoxic effects of AFB1 due to their high expression of GST alpha-3, therefore, v79h1A2/mGSTA3 was predicted to show protection. The cells expressing hCYP1A2 activated the AFB1 as evidenced by the 20-fold enhancement of cytotoxicity compared to non-expressing v79cells (IC50 = 5.4 nM vs. >100 nM in the parent cell line). Protection against cytotoxicity in cells co-expressing hGSTM1 was 1.4-fold (IC50 = 7.4 nM; not significant) but cells expressing mGSTA3 showed a 4.4-fold protection (IC50 = 24 nM). Protection by the GSTs against mutagenicity of AFB1 at the hprt locus was stronger overall than the protection against cytotoxicity, and was most evident at the highest concentration of 10 nM. Fold protection against AFB1 mutagenicity at the highest dose was 2.4-fold in cells expressing hGSTM1 and 3.6-fold in cells expressing mGSTA3. When DNA alkylation was investigated, the protection of both GSTs closely mirrored their protection against mutagenicity, with cells expressing hGSTM1 and mGSTA3 showing 2-fold and 3.3-fold protection, respectively, at 48 hrs of exposure.

***P9** MUTATIONAL AND TRANSCRIPTIONAL RESPONSE OF *SALMONELLA TO* MX: CORRELATION OF MUTATIONAL DOSE RESPONSE TO CHANGES IN GENE EXPRESSION, William O. Ward[1], Steffen Porwollik[2], Nancy Hanley[1], Sarah H. Warren[1], Michael McClelland[2], and David M. DeMarini[1], [1]Environmental Carcinogenesis Division(B143-06), US Environmental Protection Agency, Research Triangle Park, NC 27711, USA; [2]Sidney Kimmel Cancer Center, 10835 Altman Row, San Diego, CA 92121 USA

We measured the mutational and transcriptional response *of Salmonella* TA100 to 3 concentrations of the drinking water mutagen 3-chloro-4-(dichloromethyl)-5-hydroxy2(5H)-furanone (MX). The mutagenicity of MX in strain TA100 was evaluated in a 30min suspension assay, and the mutagenic potency was 615 rev/μM (r^2= 1.0). Gene expression also increased with MX concentration. At 0.46 μM and 1.15 μM, MX increased gene expression of 8 genes by ≥50%, and at 2.30 μM, MX increased expression of 39 genes by ≥50%. Among these 39 genes, 15% were involved in energy production and conversion, and 10% were involved in cell motility. Among the 6 genes that were most responsive to MX treatment, all demonstrated a monotonically increasing response to the three concentrations of MX. Three of these genes were associated with the stress response: *cpxP* specified a periplasmic protein that combats the lethal phenotype associated with the synthesis of a toxic envelope protein; *recA* binds to ssDNA and activates DNA repair; *rmf* binds 70S ribosomes to form 100S particles that suppress protein synthesis under stress conditions.

The other 3 genes *(nanH, ftsI,* and *STM0718)* do not have established connections to the stress response. *nanH* is a neuraminidase; *ftsI* is required for cell division; and *STMO718* is a putative cytoplasmic protein with no known function. These data indicate that MX, in addition to inducing mutations, also induces expression of genes involved in the SOS response as well as other genes. [Abstract does not necessarily reflect the policy of the US EPA.]

***P10** EFFORTS TO EXPAND THE DSSTOX STRUCTURE-SEARCHABLE PUBLIC TOXICITY DATABASE NETWORK, Brian A. Rogers[1] and Ann M. Richard[2], [1]EPA-NC Central Univ. Student COOP, US EPA, RTP, NC 27711; [2]Nat. Health & Environ. Research Lab, US EPA, RTP, NC 27711

A major goal of the DSSTox website is to improve the utility of published toxicity data across different fields of research. The largest barriers in the exploration of toxicity data by chemists and modelers are the lack of chemical structure annotation in the research literature and public databases coupled with the lack of full access to this toxicity data. Each DSSTox database is written with the collaboration of toxicity experts and incorporates standardized chemical structure annotation and data fields that enhance relational searching across multiple databases and can aid development of structure-activity relationship models. The DSSTox website (http://www.epa.gov/nheerl/dsstox) offers full access to toxicity database files and provides resources to encourage the scientific community to participate in the project. Efforts to expand the DSSTox project are centered on increasing the number of published toxicity databases and coordinating with other public database efforts to enhance the utility of these files. Undergraduate student EPA grantees play a prominent role in the DSSTox project, particularly in database development, annotation, and quality review. Most recently, the focus is on automating the most tedious and error prone aspects of these tasks, with scripts written in the open source programming language, Python, to be made publicly available on the DSSTox website. This project has provided an opportunity for undergraduate students to be introduced to many aspects of database development, chemical relational database programs, programming, and chemistry and toxicology aspects of public toxicity databases, while contributing to development of an important public resource. *This abstract does not reflect EPA policy.*

***P11** GESTATIONAL EXPOSURE TO THE PHYTOESTROGEN GENISTEIN INFLUENCES EPIGENETIC GENE REGULATION IN MICE, Dana C. Dolinoy[1], Robert A. Waterland[2], Randy L. Jirtle[1], [1]Department of Radiation Oncology, Duke University Medical Center, Box 3433 Durham, NC., [2]Departments of Pediatrics and Molecular Human Genetics, Baylor College of Medicine, Houston, TX

Exposure to the phytoestrogen genistein has been linked to a variety of beneficial effects, including cancer chemoprevention and decreased adipose deposition, as well as a number of negative outcomes including reduced reproductive health. Since the epigenome is particularly susceptible to dysregulation during embryogenesis, we investigated the effect of gestational exposure to moderate levels of dietary genistein in viable yellow Agouti (A^{vy}) mice. Female a/a dams were randomly assigned to receive soy-free diet or soy-free diet supplemented with 250 mg/kg diet of genistein two weeks prior to mating with A^{vy}/a males and throughout gestation and lactation. Coat color phenotype and CpG methylation of the A^{vy} intracisternal A particle (IAP)

retrotransposon were assessed in day 21 A^{vy}/a offspring. Bisulfite sequencing was used to quantify site-specific CpG methylation. Maternal supplementation with genistein shifted the coat color distribution of A^{vy}/a offspring toward the brown (pseudoagouti) phenotype. Methylation analysis at nine A^{vy} IAP CpG sites showed increased percentage of cells methylated in genistein exposed litters in comparison to controls. IAP methylation profiles in day 120 tissue derived from the endoderm (liver), mesoderm (kidney), and ectoderm (brain) were correlated to day 21 tail tissue suggesting that genistein acts early in embryonic development and that methylation effects are not transitory but persist into adulthood. These results suggest that early exposure to genistein affects adult phenotype via permanent alterations in the epigenome and may influence cancer chemoprevention and disease susceptibility.

***P12** CYTOTOXIC EFFECTS OF A NOVEL DRUG PT-ACRAMTU ON HUMAN LUNG CANCER CELL LINES, Takiyah D Starks[1] and Sue Hess[2], NCA&T, Greensboro, NC 27411 and WFUBMC, Winston Salem, NC

Lung cancer has the highest mortality rate of any type of cancer in the world. It is estimated that one million deaths occur each year worldwide from lung cancer. Cigarette smoking contributes to the majority of lung cancer cases. Chemotherapy and surgery are the standard modes of treatment options. One chemotherapeutic form of treatment is platinum-based drug therapy. Cisplatin, Carboplatin, and oxaliplatin have been successfully used in the treatment of various malignancies. Although, studies have established the survival efficacy of platinum drug therapy, use is restricted by toxicity and acquired resistance. Therefore more effective modes of drug treatments need to be evaluated. A novel drug PT-ACRAMTU has been developed in the chemistry department at the University Of Wake Forest. This hybrid compound combines features of an alkalating/metalating agent with topisomerase-based intercalation that we believe may circumvent the problem associated with platinum chemotherapy. This unusual type of DNA targeted platinum-acrdinylthiourea conjugate exhibits several unprecedented chemical and biological properties and may truly represent a new type of platinum and acridine anti-cancer drug. Therefore, we hypothesize PT-ACRAMTU will kill lung cancer cell lines. However, this may be dependent on the molecular background of the cell line. PT-ACRAMTU was observed to be cytotoxic ina variety of lung cell lines with molecular backgrounds. In addition PT-ACRAMTU does not appear to be cytotoxic to normal cells. Therefore, we believe we might have identified a drug that may be effective in lung cancer cell lines..

***P13** INHIBITION OF FRIED MEAT-INDUCED COLON CELL DNA DAMAGE: PILOT FEASIBILITY STUDY, Daniel Shaughnessy[1], Lisa Gangarosa[2], Barbara Schliebe[2], David DeMarini[3], David Umbach[4], Robert Sandler[2], Jack Taylor[1], [1]Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences, [2]Department of Gastroenterology, UNC School of Medicine, University of North Carolina at Chapel Hill, [3]Division of Environmental Carcinogenesis, US Environmental Protection Agency, [4]Department of Biostatistics, National Institute of Environmental Health Sciences

Dietary exposures have been implicated as risk factors in colorectal cancer. Such agents may act by causing DNA damage or may be protective against DNA damage. The effect of dietary exposures in either causing or preventing damage has not been directly assessed in colon tissues. In this pilot study of dietary factors and DNA damage, involving 16 healthy volunteers in

a four-week controlled feeding study, genetic damage to colonic epithelium and blood lymphocytes induced by pyrolysis products formed in cooked meat, as well as the putative protective effects of cruciferous vegetables, yogurt, and chlorophyllin against that damage was assessed using the single cell gel electrophoresis (Comet) assay. In the first phase of this pilot study, eight subjects were fed either a baseline diet or a diet high in fried meat in two-week intervals. In the second phase, the remaining eight subjects were fed either the fried meat diet or a diet containing fried meat along with the putative inhibitors. In both phases of the study, blood was drawn and rectal biopsies were obtained from subjects each week during the four-week study periods. Damage in lymphocytes and colon epithelium from the different dietary regimens was evaluated in the Comet assay, and changes in mutagenicity in urine and fecal samples were evaluated in the *Salmonella* plate incorporation assay. Results from this pilot study will be used to determine the feasibility of conducting a main study that will evaluate short-term damage associated with fried meat consumption as well as the possible protective effects of inhibitors of that damage.

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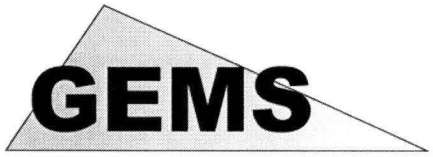

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