GEMS

Genotoxicity and Environmental Mutagen Society

Twentieth Annual Fall Meeting

"The Science of Bioterrorism"

Thursday, October 24, 2002

Friday Center
Chapel Hill, NC

GEMS
2002 Board of Directors

Genotoxicity and Environmental Mutagen Society (GEMS)

20th ANNUAL FALL MEETING
Thursday, October 24th, 2002
Friday Center, UNC, Chapel Hill, NC

"The Science of Bioterrorism"

8:00 - 8:45	Registration and Breakfast Also, career workshop roundtable for students and post-docs
8:45 - 9:00	Welcome, Amal Abu-Shakra and Diane Spencer
9:00 - 9:30	Dr. Nancy Adams, US Environmental Protection Agency **"EPA's Safe Buildings Program: Research on Building Decontamination"**
9:30 - 10:00	Dr. Paula McCready, Lawrence Livermore National Laboratory **"Biological Aerosol Sentry and Information System (BASIS)**"
10:00 - 11:30	Posters/Exhibits and Coffee
11:30 - 12:15	Competing Talks (3)
12:15 - 1:15	Luncheon Also, career workshop one-on-one with experts
1:15 - 2:00	Business Meeting/Recognition of Past Presidents
2:00 - 3:00	Competing Talks (4)
3:00 - 3:30	Break - Iced tea and cake
3:30 - 4:00	Dr. Elizabeth George, Department of Energy **"Bioterrorism and Homeland Security Research at the DOE**"
4:00 - 4:30	Awards - Talks and Posters
4:30 - 5:30	Reception

Genotoxicity and Environmental Mutagen Society

P.O. Box 13475, Research Triangle Park, North Carolina 27709

Dear GEMS Members,

Thanks to GEMS for a great couple of years. Thanks to you, the membership, for your continued support and commitment. And, thanks to our excellent President-Elect Diane Spencer, who designed our outstanding meeting on "Metabonomics" in Spring 2002. She invited Dr. Kenneth Olden, Director of the National Institute of Environmental Health Sciences (NIEHS), to open the meeting with introductory remarks on metabonomics as a component of a total scientific program; Dr. Susan Sumner (Paradigm Genetics Inc.), who gave an overview of metabonomic techniques in her lecture titled *"Introduction to Metabonomics"*; Dr. John Connelly (Metabometrix Ltd. and Research Fellow at Imperial College, London, UK), who gave a thorough lecture on "Toxicity Screening with NMR-based Metabonomics"; Mr. Scott Harrison (Paradigm Genetics Inc.), who presented a technique-oriented lecture titled: "Complete Metabolic Profiling"; and Dr. Chris Corton, who gave a thorough finale lecture on "Bridging Metabonomics and transcript profiling: system biology approach to understanding chemical action." A one-day scientific meeting could not get better than that!

Now as we turn our attention to our GEMS Fall 2002 meeting program [October 24, 2002 at the UNC Friday Center in Chapel Hill, NC], we must acknowledge Ms. Spencer for bringing us a most distinguished group of experts to present "The Science of Bioterrorism". I would like to use this platform to welcome our GEMS Fall 2002 speakers: Dr. Nancy Adams (US-EPA), Dr. Paula McCready (LLNL) and Dr. Beth George (DOE) and to express the GEMS Council's deep appreciation for their friendship and enthusiastic acceptance to present at the meeting. I would also invite nonmembers attending this excellent meeting to consider becoming members and urge members to consider taking a more active role in GEMS. The results of the elections to the GEMS council will be announced during the fall meeting. I am sure that the new council will serve enthusiastically and faithfully to safeguard the excellent standing and progress of GEMS.

Acknowledging all the present officers, council members, and (ex-officio) friends of GEMS is one of the very important and cherished final tasks for a soon-to-be Past-President. I would like to thank again and again Diane, Lance, Susan, Carl, Mark, Gloria, Lori, Lois, Keith, Marie, Steve, Jane, Barbara, Kristine, Carolyn, Ben, Heather, and last but not least Frank. Thank you Frank for our great Web announcements!

See you on October 24, 2002! Good Luck to all the students competing for Best Talk and Best Poster awards, congratulations to all their mentors for getting them to that stage of scientific knowledge and skill, and a special thanks to our generous corporate sponsors.

Amal Abu-Shakra, Ph.D.
GEMS President
North Carolina Central University
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Durham, NC 27707

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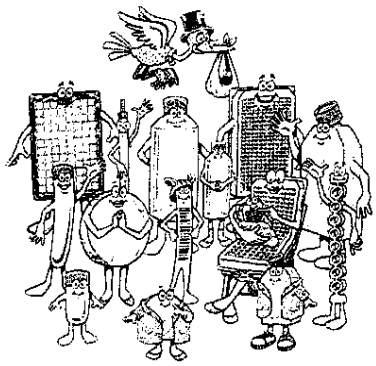

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A special thanks to NIEHS, NC Biotechnology Center and CIIT Centers for Health Research for their financial support.

Oral Presentations

***T1** EFFECTS OF BENZENE ON HEMATOPOIETIC STEM CELLS. B Faiola, ES Fuller, VA Wong, D Abernethy, L Pluta, K Roberts, L Recio and J Everitt. CIIT Centers for Health Research, RTP, NC, USA.

Benzene, a carcinogen that induces chromosomal breaks as a primary mode of genotoxicity in the bone marrow (BM), is associated with leukemia in humans. Genetic consequences resulting from benzene metabolites interacting with molecular targets and misrepair of DNA lesions can lead to changes in hematopoietic stem cells (HSC) that give rise to the leukemic clones. We used two approaches to investigate benzene-induced DNA damage responses in HSC. We compared the effects of 1,4-benzoquinone (BQ) treatment and benzene inhalation on male and female 129/Sv and 129/SvJ mice that differ at the *Prkdc* locus encoding the catalytic subunit of DNA-PK which has a primary role in nonhomologous end joining. HSC were enriched from BM by negative selection then purified by fluorescence activated cell sorting. HSC cultured *in vitro* in the presence of BQ for 24 hours showed a dose dependent cytotoxic response. Alternatively, mice were exposed to 0 or 100 ppm benzene for 6 h/day, 5 days/week for 2 weeks. Male mice of both strains had decreased white blood cell counts following benzene inhalation while female mice showed no change. Genotoxicity *in vivo* was assessed using flow cytometry to enumerate micronucleated mature normochromatic erythrocytes (MN-NCE) and micronucleated reticulocytes (MN-PCE) in blood. Although male mice were significantly more susceptible to benzene-induced genotoxicity than female mice, comparable increases in MN-NCE and MN-PCE populations in both strains of mice were seen, indicating the difference in *Prkdc* allele does not affect benzene-induced genotoxicity. There was no significant change in the fraction of apoptotic cells in BM and in the percentage of BM HSC from exposed mice compared to unexposed mice. RNA was isolated from BQ-treated HSC and HSC of exposed and unexposed mice. Quantitative RT-PCR of DNA repair genes will allow us to develop insights into the DNA repair pathway(s) that act on benzene-induced DNA lesions.

***T2** SELECTING TRANSPOSITIONS OF A LOXP SITE-CONTAINING MARKERLESS TRANSPOSON INTO GENOMIC DNA IN BACS AND PACS USING PHAGE P1 HEADFUL PACKAGING

Sushmita Mukherjee[2], Pradeep K. Chatterjee[1], Willie Wilson[2], Jr., Ken R. Harewood[1] & Goldie Byrd[2].

[1]Julius L. Chambers Biomedical/Biotechnology Research Institute, & [2]Department of Biology, North Carolina Central University, 1801 Fayetteville Street, Durham, NC 27707.

New loxP site containing Tn10 mini-transposons devoid of mammalian cell specific promoter elements were constructed for functional mapping of long-range transcription regulatory sequences in BACs. The transposons were capable of generating nested deletions from one end of the genomic DNA insert by Cre mediated recombination between the transposed lox P site and the one endogenous to the BAC clone. The panel of BAC deletions generated using such transposons should allow unambiguous mapping of long-range regulatory sequences functionally without masking those endogenous to the gene. The transposon, pTnMarkerless/loxP, uses a

novel strategy to select for insertions: instead of screening for a growth advantage conferred by the transposed piece of DNA upon the target, such as expression of antibiotic resistance, insertions into genomic DNA were indirectly selected by their ability to delete enough DNA from a large BAC so as to enable its packaging within a P1 phage head with both loxP sites intact. There is however both a desirable and an undesirable outcome to such an indirect mode of selection: 1) because the selection is not antibiotic resistance marker dependent, TnMarkerless/loxP can be used to generate nested deletions efficiently also in PAC clones, and 2) deletions through illegitimate recombination unrelated to LoxP-Cre also get packaged and recovered, and subsequent size analyses of the vector band is required to identify them. The procedure nevertheless offers a simple approach to map recombinogenic sequences in BACs.

***T3** MUTAGENICITY OF BENZO[A]PYRENE IN V79 CELLS EXPRESSING HUMAN CYTOCHROME P4501A1 ALONE OR IN COMBINATION WITH HUMAN GLUTATHIONE-S-TRANSFERASES, Mary E. Kushman[1] and Alan J. Townsend[1,2], [1]Department of Cancer Biology and Comprehensive Cancer Center, Wake Forest University School of Medicine, Winston-Salem, NC 27127, and [2]Department of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC 27157

We have used V79MZ cells stably transfected with human CYP1A1 alone or in combination with human GSTP1-1 or GSTM1-1 to study the dynamics of activation vs. detoxification of B[a]P. In this study, it was of interest to determine how effectively these human GST isoforms, in the presence of human CYP1A1, protect against mutagenicity at the *hprt* locus. CYP1A1 activity is comparable across all cell lines, while hGSTP1-1 shows a specific activity approximately twice that of hGSTM1-1. The results demonstrated that B[a]P mutagenicity is dose- and time-dependent in cells expressing CYP1A1 only. At each of the concentrations of B[a]P tested, there was a 2.5 to 3 fold protection against mutagenicity observed in the 1A1/hGSTP1-1 expressing cells compared to the 1A1-only expressors. In the 72-hour time course study including both GST isoforms, mutant colony formation developed over the first 24 hours, while it appeared to plateau over the course of 48-72 hours. Fold protection against mutagenicity at each time point was consistent with that seen in the dose-response study, and was approximately proportionate to the levels of GST expression in the cell lines. This is consistent with previous results obtained in our laboratory demonstrating that resistance to cytotoxicity of B[a]P in the 1A1/hGSTP1-1 and 1A1/hGSTM1-1 cell lines is commensurate with expression level of the GST isozyme. However, resistance to mutagenicity is significantly lower than that to cytotoxicity (2.8- and 1.4-fold resistance to mutagenicity and 15- and 8.5-fold resistance to cytotoxicity in the hGSTP1-1 and hGSTM1-1 expressors, respectively). The results indicate that hGSTP1-1 and hGSTM1-1 confer proportionally similar protection. However, the differential protection against mutagenicity and cytotoxicity suggests that these two toxic endpoints may be mediated by different metabolites of B[a]P.

***T4** SLOW REMOVAL OF THYMINE FROM N^2ETHYLG:T MISMATCHES BY HUMAN THYMINE DNA GLYCOSYLASE G. Scott Jenkins[1], Primo Schär[2], James Fishbein[3], Fred Perrino[1], and Steve A. Akman[1] Cancer Biology, Wake Forest University School of Medicine, Medical Center Blvd, Winston-Salem, N.C. 27157 [1]; University of Zürich[2]; University of Maryland, Baltimore County[3]

Human thymine DNA glycosylase (hTDG) is an enzyme that initiates base excision repair of G:T and G:U mismatches. Further studies have revealed the ability of hTDG to excise thymine from mispairs with modified guanine adducts. In this study we examined the ability of hTDG to recognize thymine mismatched with N^2-ethylguanine (N^2eG), which has been identified in the DNA of human alcoholics, and O^6-ethylguanine (O^6eG), which is formed from such alkylating agents as N-ethyl-N-nitrosourea and ethyl methanesulfonate. Purified hTDG was incubated with 3'labeled 32-bp DNA substrates containing either a unique guanine or modified guanine adduct mismatched with thymine. The substrates were then treated with 1M piperidine to cleave abasic sites and electrophoresed on 15% DNA sequencing gel. We observed almost complete excision of the substrates containing G:T and O6eG:T mismatches after incubating the substrate with hTDG for 20 minutes at 30°C. However, substrate containing N^2eG:T showed less than 8% excision after incubating with hTDG for 80 minutes at 30°C. Preliminary binding studies have shown that hTDG binds to N^2eG:T substrate but with lower affinity compared to G:T and O^6eG:T. We are currently completing kinetic and binding studies of these substrates with hTDG. The data from this preliminary study suggest that impaired post-replication repair of N^2eG may cause of mutagenicity by this adduct.

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* An asterisk by the abstract number indicates that a presenter is in competition for the Best Talk or Poster Award. The Best Talk recipient will receive up to $1500 to be used to defray costs associated with attendance at a national professional meeting of his or her choice, pending the Board's approval. There are two poster awards. The first place poster winner will be awarded $100 and the second place poster recipient will receive $50.

Poster Presentations

***P1** ROLE OF DNA POLYMERASE ETA IN CISPLATIN- AND UV-INDUCED IN VIVO MUTAGENESIS AT THE HUMAN HPRT LOCUS, N. King[1], K. Bassett[2], S. Chaney[2], M. Cordeiro-Stone[1], [1]Department of Pathology and Laboratory Medicine and [2]Department of Biochemistry and Biophysics, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599.

Unrepaired DNA damage can lead to stalled replication forks. Specialized DNA polymerases can use the damaged DNA template to catalyze translesion synthesis. DNA polymerase η (pol η) accurately and efficiently replicates past cyclobutane pyrimidine dimers, the most common UV-induced lesions. The gene encoding this polymerase is mutated in the variant form of the cancer-prone genetic disease xeroderma pigmentosum. Cultured fibroblasts lacking pol η are hypersensitive to UV-induced mutagenesis. Pol η has been shown to replicate efficiently through cisplatin adducts in vitro. We seek to determine whether pol η is a major contributor to replication past cisplatin adducts in vivo. We are comparing mutant frequencies at the human hypoxanthine-guanine phosphoribosyl transferase (HPRT) locus in diploid human fibroblasts exposed to cisplatin or UVC. Cell lines immortalized by the ectopic expression of telomerase are being used, one representing pol η-mutant fibroblasts (hTERT-2359) and another normal fibroblasts (hTERT-NHF1). Mutation of the *HPRT* gene is detected as the inability to synthesize toxic nucleotides from the purine analog 6-thioguanine (6-TG). Mutant frequency is scored by the number of resistant colonies in culture medium containing 6-TG, adjusted for colony forming efficiency in medium alone. UVC and cisplatin caused dose-dependent increases in mutant frequency in both cell lines. In cells with mutant pol η, the average mutant frequency was 3.8-fold higher than in normal cells, when both cell lines were treated with the same UVC fluence. Conversely, cisplatin-induced similar mutant frequencies in both cell lines. These results indicated that the functional absence of pol η alone does not affect the frequency of cisplatin-induced mutagenesis at the human HPRT locus in vivo. Cisplatin is a widely used chemotherapeutic agent. Understanding the mechanisms of its mutagenicity is important for the future design of highly efficient and less mutagenic anti-cancer platinum drugs. Supported by PHS awards CA55065 (MCS) and CA8440 (SGC).

P2 EXPRESSION OF MUTANT HUMAN KI-RAS INDUCES LUNG TUMORS IN BITRANSGENIC MICE, H. Floyd[1], J. Tichelaar[2], S. Dance[1], J. Everitt[4], J. Whitsett[3]; M. Miller[1] [1]Wake Forest University, Winston-Salem, NC; [2]University of Cincinnati, Cincinnati, OH [3]Children's Hospital Research Foundation, Cincinnati, OH 4CIIT, Research Triange Park, NC.

Ki-*ras* mutations have been implicated as an early event in the development of human and mouse lung adenocarcinomas. We developed a bitransgenic mouse model to regulate mutant human Ki-*ras* in a lung-specific and DOX-inducible manner. Monotransgenic mice containing the mutant Ki-*ras*CYS[12] transgene linked to a tetracycline-inducible promoter are crossed to monotransgenic mice that constitutively express the reverse tetracycline transactivator from either the lung-specific SP-C or CCSP promoters. Induction of the Ki-*ras* transgene was detected by RT-PCR in bitransgenic mice following 1 week of DOX exposure. Bitransgenic mice exhibited hyperplastic lung foci after only 12 days of DOX treatment. By 3 months of treatment,

extensive epithelial hyperplasia of the alveolar region of the lung tissue could be seen, as well as multiple macroscopic tumors, the majority of which were less than 1 mm in size. The tumor incidences following 3 months of DOX treatment were 80% and 100% for SP-C/Ki-*ras* and CCSP/Ki-*ras* mice, with tumor multiplicities of 2.0 ± 2.0 and 7.2 ± 5.8 (n=5), respectively. Histopathology analysis of one of these lesions identified it as a well differentiated adenoma, as the lesion contained normal sized nuclei and was encapsulated with no signs of invasion into surrounding tissue. These analyses are continuing on other tissue samples. Lung morphology of untreated bitransgenic mice and DOX-treated single transgenic mice was normal. These results indicate that induction of mutant Ki-*ras* in the lung is sufficient to induce the formation of lung tumors, providing strong evidence that mutation of Ki-*ras* is a critical, early event in lung tumor pathogenesis. This mouse model is being utilized to further define the role of mutant Ki-*ras* in lung tumorigenesis and should be an important new model for the testing and development of chemopreventive and chemotherapeutic agents for lung cancer.
(Supported by NCI grant CA91909)

***P3** CHEMOTHERAPEUTIC EFFECTS OF PERILLYL ALCOHOL ON LUNG CANCER CELL LINES, Mian Xu, Heather M. Smith, Suzanne M. Greth, James P. Vaughn, Kurt Lohman, Gregory L. Kucera, Mark C. Willingham, and Mark S. Miller, Wake Forest University School of Medicine, Winston-Salem, NC.

Perillyl alcohol (POH) is being tested in clinical trials as an anti-cancer agent for breast cancer. Rodent studies have shown that other organs respond to POH as well. The mechanism of action of POH has not been definitively established; POH has been shown to induce apoptosis in some tumor cell types but is cytostatic in other cancer cells, such as breast tumors. We treated 2 different lung cancer cell lines, H322 and H838, with POH to determine the anti-tumor properties of this agent. A sulforhodamine B cell proliferation assay was used to determine the effects of POH after 1 and 5 days of treatment with 0.25 mM, 0.5 mM, 0.75 mM, 1.0 mM, and 1.5 mM POH. After 1 day of treatment little difference could be seen between the control and highest concentrations of POH. However, after 5 days of treatment, H322 cells showed a dose-dependent decrease in cell proliferation ranging from 15%-83%. The H838 cell line was somewhat less responsive and showed a maximal decrease in proliferation of 70% at 1.50 mM POH. A clonogenic assay demonstrated that while there was no significant effect of POH after 1 day of exposure, a dose-dependent decrease in colony formation, ranging from 15%-97% in H322 cells and 20%-100% in H838 cells, was seen after 5 days of treatment. Using a colorimetric enzymatic assay, we observed that POH activated caspase-3 activity 3- to 6-fold. Time-lapse video microscopy revealed that apoptotic cells were evident within 24 to 48 hr of treatment with 1.5 mM POH, with apoptosis occurring earlier in the H322 than the H838 cells by approximately 20-24 hr. At the doses employed the cells appeared to enter apoptosis in an asynchronous manner, reflecting the use of clinically relevant but not maximally toxic doses of POH. The appearance of apoptotic cells coincided with the increase in caspase-3 activity and cleavage of poly (ADP-ribose) polymerase. Nuclear staining with DAPI confirmed the classical characteristics of apoptosis in POH treated cells. Our results suggest that POH may be an effective anti-cancer drug for lung cancer patients and may mediate its anti-tumor effects by stimulating apoptosis in lung tumor cells.

(MX and HMS contributed equally to the work. Supported by the Vaughn-Jordon Foundation, Inc.)

P4 EXPOSURE TO 4,4'-METHYLENEDIANILINE (MDA) IN THE HELICOPTER MANUFACTURING INDUSTRY. T. Weiß,[1] H.U. Käfferlein,[2] H. Schuster[3] and J. Angerer[1], [1]Institute of Occupational Medicine, University Erlangen, Germany, [2]CIIT Centers for Health Research, RTP, NC 27709, [3]Eurocopter GmbH Donauwörth, Germany.

4,4'-methylenedianiline (MDA) is mutagenic and carcinogenic in animal experiments. MDA is an important intermediate in the production of isocyanates. However, it is also used as cross-linking agent in epoxy hardeners, e.g., during the production of rotor blades in the helicopter manufacturing industry.
Aim of the study was (1) to determine the external and internal exposure of workers exposed to MDA in the helicopter manufacturing industry by ambient and biological monitoring, (2) to study the main routes of uptake in humans at workplace conditions and (3) to proof various safety measures in order to establish an improved protection for the employees. For this purpose, we analyzed over a period of three years (1997-2000) 194 urine samples of 18 employees occupationally exposed to MDA. Samples were taken at different time points in order to study the excretion pattern of MDA in urine. Ambient monitoring of MDA was also carried out during the same time by stationary air sampling. The influence of various protection measures on MDA levels in urine was also studied.
MDA could be identified in 76% of all urine samples. The median elimination half-life was ~7h. Depending on the exposure situation, the time of specimen collection and protection measure urinary MDA levels were determined to be between <0.3 and 80 µg/l. However, nearly all measurements of MDA in air revealed negative results (≤0.1 mg/m^3) pointing to the fact that MDA is predominantly taken up by dermal absorption. In addition, especially those protection measures aiming at the minimization of dermal uptake emerged to be the most effective.
Our study turned out that ambient monitoring completely underestimated the exposure situation of MDA at the workplace under the particular workplace conditions. Therefore, biological monitoring rather than ambient monitoring should be considered as an important part in human risk assessment of MDA.

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☐ Please check if you are a NEW MEMBER

Would you be willing to run for election as a Councilor or Officer of GEMS? Yes ☐ No ☐

Send to: *Susan Ross, Treasurer*
GEMS
P.O. Box 13475
RTP, NC 27709

☐ Please check if you need a RECEIPT

Please make checks payable to GEMS.