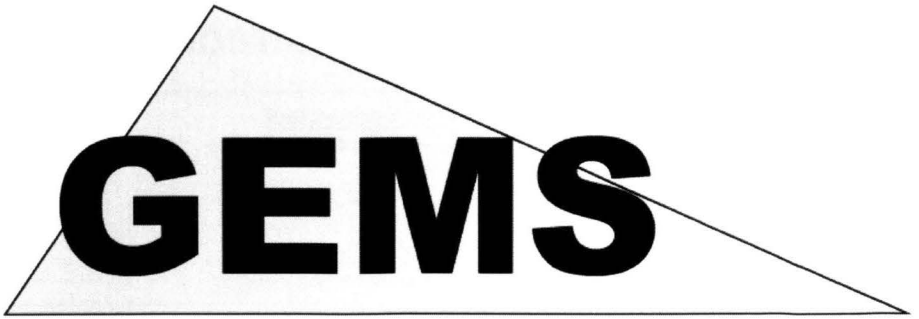

Genetics and Environmental Mutagenesis Society

2006 Spring Meeting

"Antimutagenesis and Anticarcinogenesis"

Monday
April 24, 2006

North Carolina Biotechnology Center
15 Alexander Drive
Research Triangle Park, NC

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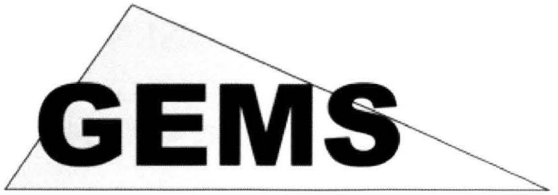

SPRING MEETING

April 24th, 2006
North Carolina Biotechnology Center

"Antimutagenesis and Anticarcinogenesis"

8:00—9:00	Registration and Continental Breakfast
9:00—9:15	Welcome: Dr. Les Recio, GEMS President Speaker Introduction: Dr. Greg Stuart, GEMS President-Elect
9:15—10:15	Dr. Rudolph I. Salganik, School of Public Health and School of Medicine, University of North Carolina at Chapel Hill *Apoptosis Against Mutagenesis and Carcinogenesis*
10:15—10:45	Coffee Break
10:45—11:30	Dr. Karen S. Katula, Department of Biology, University of North Carolina at Greensboro *Folate and Carcinogenesis: A Complex Relationship Based On Multiple Mechanisms*
11:30—12:15	Dr. Errol Zeiger, Errol Zeiger Consulting *Identification of Antimutagens and Anticarcinogens: Issues and Fallacies*
12:15—1:30	LUNCH
1:30—2:15	Dr. Alan J. Townsend, Department of Biochemistry, Wake Forest University School of Medicine *Protection Against Carcinogen Genotoxicity by Glutathione Transferases Co-Expressed with Cytochrome P-450*
2:15—3:00	Dr. Daniel Shaughnessy, Laboratory of Molecular Carcinogenesis, National Institute of Environmental Health Sciences *Dietary Antimutagens: Studies in Bacteria and Humans*
3:00—3:45	Dr. Thomas W. Kensler, Bloomberg School of Public Health, John Hopkins University *Role of Keap1-Nrf2 Signaling in Cancer Chemoprevention*
3:45—4:30	Reception

INVITED SPEAKER PRESENTATIONS

APOPTOSIS AGAINST MUTAGENESIS AND CARCINOGENESIS

Rudolph Salganik, M.D., Ph.D., School of Public Health, School of Medicine, University of North Carolina at Chapel Hill

Numerous environmental compounds are pre-mutagenic and pre-carcinogenic. Most of such compounds became mutagenic only after metabolic activation by cytochrome P450 enzymes. Such compounds are exemplified by polycyclic aromatic hydrocarbons (PAH). Modified PAH are covalently attached to DNA guanine and adenine residues. These bulky purine derivatives due to the wrong base-pairing induce numerous point mutations that can promote the development of cancer. The damaged DNA induces protective mechanisms that start from the synthesis of p53 protein. This protein promotes generation of reactive oxygen species (ROS) by mitochondria (MT) and cytochrome P450. The excessive ROS disrupt MT membrane potential ($\Delta\psi_m$) and form MT pores that make possible the release of cytochrome C, Apaf-1, and caspase-9 from MT to cytosol. These proteins form apoptosomes that activate a chain of located downstream caspases and DNase. Activated caspases digest selected cell proteins and DNA that kills targeted abnormal cells. The p53 protein activates also transcription of proapoptotic PUMA, Noxa, BAX and Bid genes. Thereby mutagenized, precancerous and cancerous cells are selectively killed by p53-induced apoptosis. ROS induce also DNA repair enzymes. The repair of damaged DNA and the apoptosis protect partially humans from cancer. However, these barriers are not strong enough to guarantee a reliable and stable protection. Since ROS serve as potent inducers of DNA repair and apoptosis, an increase of ROS concentration in cancer cells might enhance these protective mechanisms. However, the delivery of ROS to cancer cells from outside is hardly possible because of the short life span of these entities. We suggested that the increase of ROS concentration in cancer cells in experimental animals could be achieved by the temporary depletion such as dietary antioxidants. That is the way to increase ROS by depleting of their scavengers. The data are presented showing high pro-apoptotic anticancer and antimetastatic activity of elevated ROS concentration in brain and mammary tumors in transgenic mice fed the antioxidant-depleted diet.

Besides dietary compositions, the combinations of certain drugs and chemical compounds harboring pro-apoptotic properties can be applied to enhance apoptosis in cancer cells. Subjecting people that are dwelling in carcinogenic environment or prone to cancer to periodical "cleansing" of their bodies from mutagenized, precancerous and cancerous cells might prevent the development of this deadly disease in a high number of people that are at risk.

References:

Salganik, R.I. Biochemical Aspects of Ecology: Mechanisms of the Damage and Defense of Genetic Structures. In: Chemistry, Ecology and Health (ed. K.G. Ione), Nova Science Publishers, Inc., New York, 1995, pp. 31-50.

FOLATE AND CARCINOGENESIS: A COMPLEX RELATIONSHIP BASED ON MULTIPLE MECHANISMS

Karen Katula, Ph.D., Associate Professor, Department of Biology, University of North Carolina at Greensboro

Folates are essential water-soluble B vitamins that function as coenzymes primarily in reactions for the biosynthesis of certain nucleotides and methionine. Significantly, folate deficiency has been associated with numerous health problems including certain cancers. In this talk I will first provide an overview of particular studies examining the association between folate status and carcinogenesis and outline the possible molecular mechanisms underlying this apparent link including misincorporation of uracil, altered DNA methylation patterns, and elevated homocysteine. The remainder of the talk will focus on our investigation of gene expression changes in folate deficient cells. Using microarray analysis we compared transcript levels in normal human fibroblast cells (GM03349) grown in folate deficient and sufficient medium for seven days. The largest represented groups from the selected genes functioned in cell signaling, the cytoskeleton, and the extracellular matrix and included the Wnt pathway genes DKK1, WISP1 and WNT5A. Twelve selected genes were further validated by quantitative PCR. Analysis of six genes at 4, 7, 10 and 14 days indicated that the relative differences in transcript levels between folate sufficient and deficient cells increases with time for some of the genes. Seven of the 12 selected genes were detected in the human lymphoblast cell line GM02257 and of these, changes in four genes corresponded to the results with fibroblast cells. The mechanistic link between folate deficiency and changes in gene expression was explored by treating fibroblast cells with homocysteine, methotrexate, and the MEK1/2 inhibitor U0126 and measuring transcript levels of six genes. U0126 and methotrexate treatment more closely mimicked the gene expression changes in folate deficient cells, whereas homocysteine showed little similarity. The response of stably transfected DKK1 and TAGLN-luciferase promoter constructs to treatment with the compounds corresponded to the changes in transcript levels of these genes in treated human fibroblasts. These findings indicate that a subset of genes linked to the Ras and Wnt pathways are sensitive to folate deficiency and suggest an alternative mechanism for how folate deficiency leads to changes in gene expression and altered cell function. Finally, recent studies from other groups linking the Wnt and folate pathways will be considered in relationship to our results.

IDENTIFICATION OF ANTIMUTAGENS AND ANTICARCINOGENS: ISSUES AND FALLACIES

Errol Zeiger, Ph.D., Errol Zeiger Consulting, Chapel Hill, NC

There is an extensive literature on the identification of antimutagenic substances using pure chemicals or extracts of plants and other organisms. Based on definitive experiments, or by implication, many of these antimutagens are also considered to be anticarcinogens. Many of the reported studies use a reduction in the number or frequency of mutants or damaged chromosomes the indicators of antimutagenicity, regardless of other effects, such as cell death or mitotic inhibition, that the tested substances may have on the test system. Many of the substances identified as antimutagens in a specific test system are also mutagens in the same, or different, test systems. As a consequence, the literature contains studies that show certain chemicals to be antimutagens when tested against specific mutagens, and other studies that use these same antimutagenic chemicals as the reference mutagens when identifying new antimutagens. Antimutagenic chemicals fall into three categories: those that intercept the mutagen and prevent its reaction with activating enzymes or DNA; those that interfere with the metabolic activation of the chemical to its mutagenic form; and those that interfere with the cell's responses to the initial DNA damage. This talk will address the literature with respect to the identification of antimutagens and anticarcinogens, and some of the fallacies and contradictions associated with the approaches used.

PROTECTION AGAINST CARCINOGEN GENOTOXICITY BY GLUTATHIONE TRANSFERASES CO-EXPRESSED WITH CYTOCHROME P-450

Alan J. Townsend, Ph.D., Professor of Biochemistry, Wake Forest University School of Medicine, Winston-Salem, NC

Cancer is caused in part by DNA damage and resulting mutagenesis that may result from exposure to electrophiles that react with nucleophilic sites such as the nitrogen atoms in purine or pyrimidine bases. These chemicals may be intrinsically reactive or they may require activation via metabolic pathways such as the oxidations catalyzed by "phase I" cytochrome P450 (CYP) isozymes. An example of the latter is polycyclic aromatic hydrocarbons (PAH), a class of carcinogenic environmental pollutants formed during incomplete combustion of organic matter. The parent PAH compounds themselves are usually unreactive, but become toxic upon oxidative bioactivation by CYP-dependent pathways to multiple reactive species. These reactive metabolites may also serve as activated substrates for "Phase II" conjugation reactions that can yield conjugated products that are less reactive and more water soluble. While the phase I and phase II reactions have each been studied separately, there is little data on the competition between these opposing pathways in intact cells. We have constructed transgenic cell lines to study the dynamics of competition between activation versus detoxification of carcinogens and their metabolites. Stably transfected V79MZ cells expressing human cytochrome P4501A1 (hCYP1A1) alone, or expressing hCYP1A1 in combination with human glutathione *S*-transferase P1 (hGSTP1), were used to determine how effectively this GST isozyme protects against cytotoxicity, macromolecular damage or mutagenicity of B[*a*]P or its enantiomeric dihydrodiol metabolites (+)-benzo[*a*]pyrene-7,8-dihydrodiol ((+)B[*a*]P-7,8-diol) and (-)-benzo[*a*]pyrene-7,8-dihydrodiol ((-)-B[*a*]P-7,8-diol). Expression of hCYP1A1 resulted in 27-fold enhancement of B[*a*]P cytotoxicity (i.e. 27-fold decreased IC_{50}). in comparison to the V79MZ parent cell line that was devoid of CYP activity. Co-expression of hGSTP1 with hCYP1A1 resulted in 16-fold reduction of toxicity (i.e. 16-fold increased IC_{50}). Mutagenicity of B[*a*]P at the *hprt* locus was dependent on expression of the transfected hCYP1A1, and was reduced by only 3- to 4-fold in cells further modified to co-express hGSTP1, compared to cells expressing hCYP1A1 alone. Expression of hGSTP1 reduced adducts in total cellular macromolecules (primarily protein) by 4-fold, which correlated with the reduction in B[*a*]P mutagenicity. However, total B[*a*]P metabolites bound to DNA isolated from cells incubated with [3H]-B[*a*]P were reduced by less than 30% by GSTP1 expression. Nevertheless, ^{32}P-postlabeling analysis demonstrated nearly total elimination of the known B[*a*]P-DNA adduct, N2-guanine-BPDE , in cells co-expressing hGSTP1. This adduct, thought to be the most mutagenic of the stable B[*a*]P adducts, accounts for 15% or less of the total DNA adducts observed. These results indicate that the strong reduction in hCYP1A1-mediated B[*a*]P mutagenesis by hGSTP1 is likely due largely to prevention of the N2-guanine-BPDE adduct. However, the significant fraction (30-40%) of this mutagenesis, and the majority (> 75%) of the total DNA binding that are not prevented, together suggest formation by hCYP1A1 of a subset of mutagenic metabolites of B[*a*]P that are not effectively detoxified by hGSTP1. Further, these examples and other results to be presented indicate that the protection by GST expression against reactive species can be widely variable for different biological end-points under identical conditions of exposure.

DIETARY ANTIMUTAGENS: STUDIES IN BACTERIA AND HUMANS

Daniel Shaughnessy, Ph.D., Laboratory of Molecular Carcinogenesis, NIEHS, RTP, NC

Exposure to both mutagens and antimutagens in the diet may play a role modulating cancer risk in humans. Dietary antimutagens, especially those that inhibit both spontaneous and induced mutations, may act through a variety of mechanisms, including physical binding of mutagens, antioxidant effects, induction of Phase 2 enzymes, or modulation of DNA replication and repair pathways. We have investigated the antimutagenic effects of two food flavorings, vanillin (VAN) and cinnamaldehyde (CIN) on spontaneous mutation frequency in *Salmonella* TA104, in *E coli lacI* strains with varying DNA repair backgrounds and in the human colon cancer cell line HCT116 at the *HPRT* locus. VAN and CIN reduced the spontaneous mutant frequency by approximately 50% in *Salmonella* TA104 and in the *E. coli* strains NR9102 (wild type), NR11634 (*uvrB*), and NR11475 (*recA430*). In contrast, both VAN and CIN failed to inhibit spontaneous mutation in the recombination-deficient strain NR11317. Thus, the antimutagenic effect was dependent on recombinational repair but was independent of SOS and nucleotide excision repair. In addition, we showed that VAN and CIN were effective antimutagens against spontaneous mutation at *HPRT* in the mismatch repair-deficient (MMR^-) human colon cancer cell line HCT116, reducing spontaneous mutations by 78% and 66% at nontoxic concentrations of VAN and CIN, respectively, during 3-week exposures. Exposure to VAN and CIN for 4h also decreased mutations at *HPRT* by ~35%. However, exposure to these concentrations of VAN and CIN produced slight increases in DNA damage in the comet assay and resulted in differential transcription of genes in the MAPK pathway associated with damage response and apoptosis. We propose that VAN and CIN induce a type of DNA damage that elicits recombinational repair (but not nucleotide excision repair, SOS repair, or mismatch repair), resulting in an overall reduction in DNA damage and, consequently, a reduced spontaneous mutant frequency.

Dietary components have been implicated as risk factors in colorectal cancer in humans. Such agents may act by causing DNA damage or may be protective against DNA damage. However, the effects of dietary exposures in either causing or preventing damage have not been assessed directly in human colon tissues in vivo. We have conducted a pilot study in which 16 healthy volunteers were enrolled in a 4-week controlled feeding study. In a crossover design, eight of the subjects were fed diets for two-week periods that contained meat cooked either at high temperature, resulting in high levels of heterocyclic amines (HCAs), or at low temperature, resulting in undetectable levels of HCAs. The remaining eight subjects were fed either the high-temp. meat diet or a diet containing the high-temp. meat along with the antimutagens cruciferous vegetables, yogurt, and chlorophyllin tablets. Each week during the study, blood and rectal biopsies were obtained from the subjects. The effects of the different diets on DNA damage in colonic epithelium and lymphocytes were assessed using the comet assay, and changes in urine mutagenicity were evaluated in the *Salmonella* plate-incorporation assay in strain YG1024 + S9 mix. For subjects consuming the high- or low-temp. meat diets, Tail Moment values in the comet assay were higher in colon epithelia from patients consuming high-temp. meat diet compared to those consuming the low-temp. meat diet; however, the overall difference between the low- and high-temp. meat diets was not statistically significant. Tail Moment values were significantly lower in subjects consuming the high-temp. meat plus antimutagens compared to those consuming the high-temp. meat alone ($p = 0.026$). Urine mutagenicity increased in subjects consuming the high-temp. vs. the low-temp. meat diet. For subjects consuming the inhibitor diet, unconjugated urine mutagenicity was decreased, whereas conjugated mutagenicity was increased. Our data suggest that these dietary antimutagens may reduce the levels of DNA damage in the target tissue (colon) and alter systemic levels of genotoxicants due to consumption of a mutagenic diet.

ROLE OF KEAP1-NRF2 SIGNALING IN CANCER CHEMOPREVENTION

Thomas W. Kensler, Ph.D., Department of Environmental Health Sciences, Johns Hopkins Bloomberg School of Public Health, Baltimore, MD

The development of *nrf2* knockout mice provided the first key insights into the toxicological importance of this transcription factor signaling pathway. As examples, *nrf2* knockout mice are more sensitive to the hepatotoxicity of acetaminophen, the pulmonary toxicity of butylated hydroxytolene or hyperoxia, and the neurotoxicity of 3-nitropropionic acid. Carcinogenicity of benzo[*a*]pyrene and *N*-nitrosobutyl-(4-hydroxybutyl)-amine is exacerbated in the forestomach and bladder, respectively, of knockout mice. Finally, chronic exposure of *nrf2* knockout mice to cigarette smoke leads to enhanced development of emphysema in the lungs while disruption of *nrf2* enhances susceptibility to severe airway inflammation and asthma following allergen challenge. Genomic, proteomic and biochemical analysis indicate that *Nrf2* regulates the expression of phase 2 and antioxidative genes, as well as those affecting glutathione homeostasis, NADPH generation, solute transporters, and proteasome function. Collectively, *Nrf2*-regulated genes govern a broad-based adaptive response to environmental stresses. Nrf2 turnover and distribution in the cell is controlled by Keap1, a cysteine-rich chaperone. These cysteines of Keap1 appear to be modified by a number of cellular stresses (endoplasmic reticulum stress, oxidative stress, and shear stress), signaling molecules (nitric oxide, growth factors) and oxidized lipids (15-deoxy-$\Delta^{12,14}$-prostaglandin J_2, oxidized low density lipoproteins) leading to increased stability and nuclear accumulation of Nrf2. Of great relevance to disease prevention, this pathway also can be induced by an expanding array of small molecule drugs and natural products including dithiolethiones, isothiocyanates and triterpenoids. These compounds are potent anticarcinogens in animal models and their efficacy is lost in *nrf2* knockout mice, highlighting the central importance of this pathway. Several of these inducers are currently being evaluated in clinical trials for cancer prevention.

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