Genetics and Environmental Mutagenesis Society

29th Annual Fall Meeting

"Oxidative Stress – Role of Metals and Metalloids in Cancer"

Tuesday, November 8, 2011

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Contents

Dear GEMS Members,

Welcome to the 29th Annual Fall Meeting of GEMS. The theme of today's meeting is "Oxidative Stress – Role of Metals and Metalloids in Cancer", which is organized and chaired by our president-elect Nagu Keshava. She has done an excellent job of bringing in invited speakers on a theme that is of continued interest to the GEMS members, environmental causes of oxidative stress leading to cancer. This theme is complementary to the spring meeting that Nagu organized on the topic of understanding chemical mechanisms of action via application of genomics data. Congratulations to Nagu for organizing these two GEMS meeting and for her new role as GEMS President in the upcoming year.

I wanted to take this opportunity to thank our Corporate Sponsors, who provide financial support that is so vital to the continued success of GEMS. Many of the Corporate Sponsors have been long time supporters of our organization. I would like for every member and guest attending the meeting today to take the time to visit each of the corporate sponsor tables that display new products and lab equipment. **Please visit with each of the corporate sponsor representatives during the morning break to discuss your laboratory needs and products of interest and thank them for their continuing support.**

Organizing the Fall meeting requires the efforts of many dedicated volunteers. A special thanks to all the GEMS Board members would have faithfully attended board meetings and provided valuable advice and service. I would like to thank the outgoing Councilors Stephanie Smith-Roe and Perpetua (Peppy) Muganda, outgoing student member Stephen Roberts and current councilors Tom Hughes, Jorge Muniz Ortiz, Rebecca Fry, Peter Schad, Barbara Shane and Bill Ward. Additional thanks to Bill Ward for acting as the GEMS webmaster this past year and to Stephanie Smith-Roe for chairing the election committee. Special thanks goes to GEMS secretary Jayne Boyer and GEMS treasurer Cindy Innes for the many hours of service provided to GEMS. There are a number of other people who deserve special recognition for their constant efforts on behalf of GEMS. These include JD Wilson who is the Corporate Sponsor Representative, Carolyn Harris, who has maintained our membership list for several years and Kristine Witt, who every year volunteers to order the awards for presentations and outgoing board members.

The primary purpose of GEMS is to provide a local organization to promote the advancement of science, provide a convenient local forum for students and post doctoral students to interact with local scientists on shared interests and thus advance their careers. The meetings encourage junior scientists to present their current research, with the chance to win awards for best poster in each of the 3 categories – students, post-docs and technicians. The best platform presentation by a competing junior scientist can win a travel award of $1500 that can be applied towards the costs of attending and presenting their work at a national scientific meeting. GEMS encourage all qualified members to consider presenting at the next meeting or to encourage their students/post-docs to present at future meetings. GEMS is also a great training

opportunity for future national scientific society leaders. Consider volunteering as a candidate for an elected position in GEMS next year and gain valuable leadership experience.

I have enjoyed serving GEMS in many capacities on the Board in the last 12 years as Treasurer and Councilor. It has been an honor and a privilege to serve as your President-elect and President.

Steve Little
GEMS president, 2010-2011.

President-Elect's Message

Dear GEMS Members, Postdocs, Students and Guests,

With great pleasure, I welcome you to an exciting 29th Annual Fall Meeting of the Genetics and Environmental Mutagenesis Society. The theme of this meeting is **"Oxidative Stress – Role of Metals and Metalloids in Cancer"**. This meeting will feature the characteristics of metals and/or metalloids, their extensive metabolism involving oxidation status and their capacity to produce free radicals and hence have a role in cancer. The presentations will be made by established researchers in the area of oxidative stress. Opportunities are provided for younger researchers to showcase their work in this area.

Oxidative stress represents an "imbalance between the production and manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage". Any changes in this normal redox state of a tissue can cause toxic effects. Metals can generate reactive oxygen species and can cause modification to DNA bases, enhance lipid peroxidation and alter calcium and sulfhydryl homeostasis leading to metal-induced toxicity and carcinogenicity. **Dr. David Thomas**, the first speaker from U.S. Environmental Protection Agency, will emphasize the mechanistic basis for arsenic's actions as a toxicant and a carcinogen. Dr. Thomas will discuss arsenic metabolism, factors that modify arsenic methylation that probably contributes to inter- and intraspecies differences in susceptibility to arsenic-induced diseases. The second speaker, **Dr. Michelle Hooth** from the National Institute of Environmental Health Sciences will focus on another metal, hexavalent chromium. Dr. Hooth will discuss if oxidative damage plays a role in the carcinogenicity of hexavalent chromium based on the results of a 2-year animal bioassay conducted by the National Toxicology Program. She will also discuss the multiple potential pathways of Cr(VI)-induced genotoxicity and carcinogenicity. Our third speaker, **Dr. Adriana Oller** from the Nickel Producers Environmental Research Association, will be the final invited speaker by emphasizing the bioavailability of nickel ions and their indirect genotoxic effects on nickel carcinogenicity. She will discuss several potential modes of action including the effects of oxidative stress resulting from inflammation, macrophage activation or direct generation of reactive oxygen species by Ni (II). After lunch, a series of presentations will be made by younger scientists, including post docs and students, on their research.

I am confident that these presentations will provide an in-depth analysis of the role of metals and metalloids in cancer through an oxidative stress mechanism. I look forward to seeing you all at the meeting.

Best Wishes,

Nagu Keshava
GEMS President-elect

GEMS

Genetics and Environmental Mutagenesis Society

Oxidative Stress – Role of metals and metalloids in Cancer

8:00 AM - 8:45 AM	Registration and Continental Breakfast
8:45 AM - 9:00 AM	Welcome: Stephen Little, M.Tox., GEMS President Introductions: Nagu Keshava, Ph.D., GEMS President-elect
9:00 AM - 9:45 AM	**Linking arsenic metabolism and toxic effects** *David Thomas, Ph.D., National Health and Environmental Effects Research Laboratory, Environmental Protection Agency*
9:45 AM - 11:30 AM	Posters, sponsor exhibits and coffee
11:30 AM - 12:15 PM	**Does oxidative damage play a role in the carcinogenicity of hexavalent chromium?** *Michelle Hooth, Ph.D., National Institute of Environmental Health Sciences, National Institute of Health*
12:15 PM - 1:45 PM	LUNCH (short business meeting during lunch, election results, sponsors)
1:45 PM - 3:00 PM	Talks by post docs/students
	1:45 – 2:00 R. Prasad (Abstract T1) **2:00 – 2:15 Y. Yang (Abstract T2)** **2:15 – 2:30 J. Stumpf (Abstract T3)** **2:30 – 2:45 J. Muñiz Ortiz (Abstract T4)** **2:45 – 3:00 K. Krishna (Abstract T5)**
3:00 PM - 3:45 PM	**Nickel carcinogenicity: bioavailability of nickel ions and indirect genotoxic effects** *Adriana Oller, Ph.D., Nickel Producers Environmental Research Association*
3:45 PM	Announcement of Awards
4:15 PM	Adjourn

Invited Speakers

David Thomas, Ph.D

Senior Biologist
Pharmacokinetics Branch - Mail Drop B 105-03
Integrated Systems Toxicology Division
National Health and Environmental Effects Research Laboratory
U.S. Environmental Protection Agency
109 Alexander Drive
Research Triangle Park, North Carolina 27711

David J. Thomas is a Research Toxicologist in the Pharmacokinetics Branch, Integrated Systems Toxicology Division, National Health and Environmental Effects Research Laboratory, U.S. Environmental Protection Agency, in Research Triangle Park, NC. He received a B.S. in Zoology from Trinity College of Duke University in 1972, and a Ph.D. in Toxicology from the University of Rochester in 1978. He was a post-doctoral fellow in the Department of Pathology at the University of North Carolina at Chapel Hill from 1977 to 1980. He was assistant laboratory director of the childhood lead program at the Kennedy (now Kennedy-Kreiger) Institute in Baltimore and an assistant professor in the Department of Pediatrics in the Johns Hopkins University School of Medicine and the Department of Pediatrics in the University of Nebraska, School of Medicine. He joined the Pharmacokinetics Branch in 1991. His research focuses on the metabolism and disposition of metals and metalloids.

Presentation Abstract:

Linking Arsenic Metabolism and Toxic Effects
David Thomas, Ph.D., NHEERL, Environmental Protection Agency

The mechanistic basis for arsenic's actions as a toxicant and a carcinogen are not well understood. Arsenic undergoes extensive metabolism involving changes in oxidation state and formation of methyl-arsenic bonds; these processes affect the kinetic behavior of arsenicals and likely account for the spectrum of toxic effects associated with chronic exposure to inorganic arsenic. Thus, the factors that modify capacity for arsenic methylation probably contribute to inter- and intraspecies differences in susceptibility to arsenic-induced diseases. Recent research has demonstrated that arsenicals are readily interconverted between oxy- and thio-forms. This conversion adds a new dimension to studies of the kinetic behavior and toxicity of arsenicals and complicates dosimetric and mode of action studies. Integrating the study of the metabolism of arsenic into dosimetric and mode of action studies will better define critical steps by which arsenicals produce the changes that cause cell death or transformation.

Michelle Hooth, Ph.D.

Senior Toxicologist
Toxicology Branch
National Institute of Environmental Health Sciences
Mail Drop K2-13
Research Triangle Park, North Carolina 27709

Dr. Michelle J. Hooth is a toxicologist in the Toxicology Branch, Division of the National Toxicology Program, National Institute of Environmental Health Sciences. She is the group leader for the General Toxicology Group. She received a B.S. in Biological Sciences from Michigan State University and a Ph.D. in Toxicology from the University of North Carolina at Chapel Hill. She completed post-doctoral training in the Environmental Carcinogenesis Division, National Health and Environmental Effects Research Laboratory, U.S. EPA. She is a Diplomate of the American Board of Toxicology. Her primary responsibilities include the design, interpretation and reporting of toxicity and carcinogenicity studies for the National Toxicology Program. She also coordinates preparation of the NTP technical reports.

Presentation Abstract:

Does oxidative damage play a role in the carcinogenicity of hexavalent chromium?

Michalle J Hooth, Ph.D., NTP, National Institute for Environmental Health Sciences

Hexavalent chromium (Cr(VI)) is a contaminant of water and soil and is a human lung carcinogen. Trivalent chromium (Cr(III)) is a proposed essential element and is ingested by humans in the diet and in dietary supplements such as chromium picolinate. The NTP recently demonstrated that Cr(VI) is also carcinogenic in rodents when administered in drinking water as sodium dichromate dihydrate (SDD), inducing neoplasms of the oral cavity and small intestine in rats and mice, respectively. In contrast, there was no definitive evidence of toxicity or carcinogenicity following exposure to Cr(III) administered in feed as chromium picolinate monohydrate (CPM). Tissue distribution studies in additional groups of male rats and female mice demonstrated higher Cr concentrations in all tissues following exposure to Cr(VI) compared to controls and Cr(III) exposure. Although Cr(VI) is genotoxic in a number of in vitro and in vivo test systems, the mechanisms of genotoxicity and carcinogenicity are not fully understood. Cr(VI) readily enters cells via nonspecific anion channels, in contrast to Cr(III) which cannot easily pass through the cell membrane. Thus, extracellular reduction of Cr(VI) to Cr(III), which occurs primarily in the stomach, is considered a mechanism of detoxification, while intracellular reduction is thought to be a mechanism of genotoxicity and carcinogenicity. Cr(VI) has been postulated to exert its genotoxic effects indirectly, in part, through the generation of reactive oxygen species formed during the intracellular reduction of Cr(VI) through the more reactive Cr(V) and Cr(IV) valences to Cr(III). Other studies raise questions about the relative contribution of this mechanism. In addition, Cr(III) has been shown to interact directly with DNA and

other macromolecules to induce chromosomal alterations and mutational changes. The multiple potential pathways of Cr(VI)-induced genotoxicity and carcinogenicity will be discussed.

Adriana Oller, Ph.D.
Senior Toxicologist
NiPERA, Inc.
2525 Meridian Parkway, Suite 240
Durham, North Carolina 27713

Adriana Oller is originally from Argentina where she obtained a Master's degree in Biochemistry from Buenos Aires University in 1980. In 1983 after completing a two-year residency program in Toxicology and Forensic Chemistry (Buenos Aires University), Adriana moved to the United States where she completed a Ph.D. in Genetic Toxicology at the Massachusetts Institute of Technology in 1989. Her Ph.D. thesis examined the contribution of oxidative damage to spontaneous mutations. In 1989 Adriana moved to North Carolina where she continued genetic toxicology research on spontaneous mutations and DNA repair at the Lineberger Cancer Research Center in Chapel Hill and later on at NIEHS in Research Triangle Park. In 1994, she joined the staff of the Nickel Producer Environmental Research Association (NiPERA), where she remains today as senior toxicologist and manager for its mutagenicity and carcinogenicity research program. From 2003-2005 Adriana co-edited the Metals in Perspective column of JEM. Adriana is also a diplomat of the American Board of Toxicology. She has several peer reviewed publications on nickel.

Presentation Abstract:

Nickel carcinogenicity: bioavailability of nickel ions and indirect genotoxic effects
Adriana Oller, Ph.D., D.A.B.T, Nickel Producers Environmental Research Association

Workers' exposure to nickel-containing aerosols encountered during the processing and refining of sulfidic nickel ores has been associated with increased risks of respiratory cancer. Animal studies with individual nickel substances have demonstrated different carcinogenic potential and potency to induce lung tumors depending on the various chemical forms of nickel (e.g., no tumors induced by soluble nickel compounds and metal powder, big differences in potency between nickel subsulfide and nickel oxide). In addition, the response of different rodent species to the effects of nickel has been quite different (positive response only in rats). Can mode of action information help us reconcile the different findings in animal and human studies as well as explain the differences in rodents' response? At a recent workshop of nickel experts (http://www.tera.org/Peer/NiBioavailability), the bioavailability model for cancer induction by nickel was discussed and possible indirect effects of nickel that may contribute to its mode of action were analyzed. The panel attributed the carcinogenesis of nickel compounds to freely available nickel ion (i.e., nickel ion not bound to proteins) at the target cellular sites. While the mechanism of action is not known, the panel agreed unanimously that nickel does not act by direct DNA reactivity. Several potential modes of action were considered including the effects of oxidative stress resulting from inflammation, macrophage activation or direct generation of reactive oxygen species by

Ni (II). There is evidence for and against a prominent role of oxidative stress in nickel carcinogenesis. Work is ongoing to identify the main mechanism(s) that contribute to the carcinogenicity of nickel subsulfide in rats and the similarities and differences between gene expression patterns generated in lung cells after inhalation of nickel sulphate or nickel subsulfide. This research project includes parallel studies in air-liquid interface systems with rat and human lung cells, as well as in vivo studies in rats.

T1

Oxidative Stress, Inflammation, and DNA Damage Responses Elicited by Silver, Titanium Dioxide, and Cerium Oxide Nanomaterials

Raju Y. Prasad[1], Kathleen Wallace[2], Carl F. Blackman[2], David M. DeMarini[2], Steven O. Simmons[2]
[1] Student Services Contractor, Integrated Systems Toxicology Division, US EPA, RTP, NC 27711
[2] Integrated Systems Toxicology Division, US EPA, RTP, NC 27711

Previous literature on the biological effects of engineered nanomaterials has focused largely on oxidative stress and inflammation endpoints without further investigating potential pathways. Here we examine time-sensitive biological response pathways affected by engineered nanomaterials using a battery of stable luciferase-reporter cell lines in HepG2 cells. We measured the activation of five key stress responsive transcription factors, ELG-1, NFκB, Nrf2, AP-1 and the human promoter *IL-8,* by exposure to six titanium dioxide (nano-TiO_2) with rutile, anatase, and rutile/anatase crystal structures, two cerium oxide (nano-CeO_2), and two citrate-capped silver (nano-Ag) nanomaterials. Exposure concentrations ranged from 1-100 μg/ml for nano-TiO_2 and nano-CeO_2 and 0.1-30 μg/ml for nano-Ag. Cells were exposed for 6, 16, and 24 h to each nanomaterial. Cytotoxicity was measured in parallel using the MTT assay for nano-TiO_2 and nano-CeO_2 and the Alamar Blue assay for nano-Ag. Dynamic light scattering was used to determine the size and zeta potential of the nanomaterials in medium. Nano-Ag 10 nm citrate elicited a ~12-fold increase in ELG-1 at 1 μg/ml after 24 h. When exposed to 100 ug/ml of 10 nm anatase nano-TiO2 for 24 h, NFkB transcriptional activation increased ~2.5 fold. Nrf2 transcriptional activation increased by one nano-CeO_2, showing ~1.5 fold activation at 100 μg/ml after 24 h exposure. Anatase/rutile nano-TiO2 elicited a ~1.3 fold increase in AP1 at 1 μg/ml after 24 h exposure. Our results demonstrate the potential for engineered nanomaterials to elicit cellular stress responses, inflammation and DNA damage. [Abstract does not necessarily reflect the policies of the U.S. EPA.]

NOTES:

T2

Novel Role for E3 Ubiquitin Ligase Rad18 in Tolerance of Oxidative DNA Damage during the G1 Phase of the Cell Cycle

Yang Yang and Cyrus Vaziri
Department of Pathology and Laboratory Medicine
University of North Carolina, Chapel Hill, NC

Rad18 is an E3 ubiquitin ligase that mono-ubiquitinates Proliferating Cellular Nuclear Antigen (PCNA, a DNA polymerase processivity factor) at stalled replication forks in cells that acquire DNA damage. Mono-ubiquitinated PCNA promotes the recruitment of specialized Y-family Trans-Lesion Synthesis (TLS) DNA polymerases to damaged DNA during S-phase. TLS polymerases permit DNA synthesis on damaged DNA templates and constitute an important mechanism of DNA damage tolerance in eukaryotic cells. The mechanism(s) of Rad18 activation are poorly understood, but are thought to be triggered by DNA structures generated at stalled replication forks. Using synchronized Human Dermal Fibroblasts (HDF) we have investigated the cell cycle-dependence of PCNA mono-ubiquitination. Surprisingly, we show that various genotoxins including ultraviolet (UV) radiation and H2O2 induce robust Rad18-dependent PCNA monoubiquitination during G0 and G1. These results indicate the existence of a novel replication fork-independent mechanism of Rad18 activation. We investigated the significance of H2O2-induced Rad18 activation during G1. H2O2 treatment during G1 led to increased phosphorylation of the ATM checkpoint kinase in Rad18-depleted HDF, indicating the persistence of DNA damage due to Rad18-deficiency. These results suggest important new roles for Rad18 in the repair of DNA damage acquired during G1. Experiments are underway to determine the replication-independent mechanism(s) of Rad18 activation and to identify the putative Rad18-dependent DNA repair pathways that mediate genome maintenance in response to oxidative DNA damage during G1.

NOTES:

T3

Disease-associated *POLG* mutations cause a dominant increase in DNA damage-induced mtDNA point mutagenesis

Jeffrey D. Stumpf and William C. Copeland
National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC

Mitochondrial DNA (mtDNA) encodes proteins essential for ATP production. Loss of mtDNA maintenance caused by mutant variants of the mtDNA polymerase (encoded by *POLG*) causes mutagenesis that contributes to aging, genetic diseases, and sensitivity to environmental agents. We measured defects of mtDNA replication and increased mutagenesis caused by disease-associated mutations of the *S. cerevisiae POLG* homolog, Mip1, using two simple genetic techniques: screening for mitochondrial defective yeast that cannot grow on glycerol and selecting for base substitutions that confer erythromycin resistance. We determined that several disease-associated mutations caused mutagenesis in strains that contain both mutant and wildtype Mip1, but these mutant variants could not fully replicate mtDNA *in vivo* or reduce polymerase fidelity *in vitro*. We tested the hypothesis that polymerase mutant variants stall replication forks, resulting in error-prone bypass of mutagenic base damage by determining the sensitivity of mutant strains to the base-alkylating agent, MMS. Strikingly, MMS increased mtDNA mutagenesis up to 150-fold in several *mip1* mutants but did not affect wildtype strains nor DNA binding defective mutants, suggesting that the mechanism for damage-induced mutagenesis involves participation of the mutant polymerase at the replication fork. MMS exposure induced a subset of transversions that suggests that MMS causes error-prone bypass of methylated cytosines. These results reveal novel gene-environment interactions in mitochondrial disease that may contribute to the wide variation of age of onset among patients with similar *POLG* mutations and suggest that heterozygous carriers of disease-causing mutations may be sensitive to DNA-damaging environmental agents, leading to acquired mitochondrial toxicities.

NOTES:

T4

A genetic approach to elucidate the genotoxic pathway of monomethylarsonous acid (MMA^{III}) suggests a key role for catalase

Jorge G. Muñiz Ortiz, Kathleen A. Wallace, and Andrew D. Kligerman
US Environmental Protection Agency, Office of Research and Development, National Health and Environmental Effects Research Laboratory, Research Triangle Park, NC 27711

Arsenic-contaminated drinking water causes cancer, neuropathy, respiratory effects, diabetes, and cardiovascular disease. Its exact mode of action (MOA) is not fully understood. Oxidative stress has been proposed as a key event in the toxic MOA of arsenic. Our studies are centered on identifying a reactive oxygen species involved in the genotoxicity of arsenic using a catalase (*Cat*) knockout mouse model, which is impaired in its ability to break down hydrogen peroxide (H_2O_2) leading to an increase in hydroxyl radicals (•OH). We assessed the induction of DNA damage using the comet assay following exposure of $Cat^{+/+}$ and $Cat^{-/-}$ primary mouse splenocytes to monomethylarsonous acid (MMA^{III}) to identify the potential role of H_2O_2 in arsenic's toxicity. $Cat^{-/-}$ splenocytes are more susceptible to MMA^{III} than the $Cat^{+/+}$ splenocytes by a small (1.5-fold), but statistically significant margin. In mice splenocytes have one-third of the liver catalase activity. Therefore, comet assays were performed on MMA^{III}-treated primary $Cat^{+/+}$, $Cat^{+/-}$, and $Cat^{-/-}$ hepatocytes to determine if the $Cat^{-/-}$ cells were more susceptible to the treatment. Indeed $Cat^{-/-}$ hepatocytes exhibited higher levels of DNA strand breakage than the $Cat^{+/+}$ and $Cat^{+/-}$ hepatocytes. Our results suggest that catalase is involved in protecting cells against MMA^{III}-generated H_2O_2, and that •OH might be involved in its genotoxic pathway. Furthermore, individuals who harbor genetic polymorphisms in their catalase gene could exhibit enhanced susceptibility to arsenic toxicity. [This is an abstract or proposed presentation and does not necessarily reflect EPA policy.]

NOTES:

T5

Assessing Toxicities of Regulated and Unregulated Disinfection By-products in Normal Human Colon Cells.

Kavya Krishna[1] and Anthony DeAngelo,
[1]Centennial High School, Ellicott City, MD 21042

The presence of over six hundred disinfection by-products (DBPs) and less than half of the total organic halides present in finished water has created a need for short-term in vitro assays to address toxicities that might be associated with human exposure. . We are using a normal human colon cell culture to test individual DBPs and real-world DBP mixtures for cytotoxicity and genotoxicity capability. *Test System*: NCM460 cells, a mixed culture of human colon mucosal cells stained positive for the epithelial cell markers and negative for endothelial cell and lymphocyte markers. The cells grew attached and in suspension and possessed the capability for differentiation, which indicated a stem cell component. The cells exhibited cytochrome P450 and GSTT1-1 activities comparable to those measured in the rat large intestine. *Cytotoxicity Assay*: Approximately 50 regulated and unregulated DBPs from the chemical classes trihalomethanes, haloacetic acids, halonitromethanes, haloacetonitriles, haloacetamides, N-nitrosamines were tested for cytotoxicity in the growth inhibition assay (GIA). *General findings:* For any halogenated DBP class, iodinated species and monohalogenated DBPs were the most potent. The regulated trihalomethanes and haloacetic acids, (~70% of DBPs in chlorinated water) were less cytotoxic than the corresponding nitrogen containing DBPs, which occur at higher levels in chloraminated waters. The N-nitrosamines displayed a relatively low order of cytotoxicity. Statistically significant concordances were obtained when potencies (IC_{50}) were compared to those observed in cytotoxicity assays for mouse embryo, mammalian stem cell, and Chinese hamster ovary cell cultures. (This is an abstract of a proposed presentation and does not necessarily reflect the opinion of the US EPA.)

NOTES:

P1

Contributions of the Replication Fork Protection Complex, Timeless-Tipin, to Multiple Genome Maintenance Functions

Stephanie L. Smith-Roe, Shivani S. Patel, Ying Chun Zhou, Dennis A. Simpson, Kathleen A. Kaiser-Rogers, Marila Cordeiro-Stone, William K. Kaufmann; Department of Pathology and Laboratory Medicine, University of North Carolina at Chapel Hill, Chapel Hill, NC

Replication forks constantly encounter challenges – from chemical modifications of DNA to intrinsic characteristics of DNA sequence and metabolism – yet genomes are replicated with tremendous accuracy. The Timeless-Tipin complex interacts with components of the replication machinery (MCM2-7 helicase, replicative polymerases), checkpoint proteins (RPA, Claspin), and cohesin ring subunits, indicating a role for Timeless-Tipin in replication-dependent genome maintenance. Although we and others have shown that Timeless, Tipin, and Claspin work together to mediate ATR-dependent activation of the Chk1 kinase in the intra-S checkpoint response to DNA damage, these proteins have different contributions to normal DNA replication and genome maintenance, as do ATR and Chk1, in normal human fibroblasts (NHF). Depletion of Timeless, Tipin, and Chk1 results in cessation of DNA replication throughout S phase, which was not as apparent with depletion of Claspin, and not detected with depletion of ATR. Reduced incorporation of BrdU in Timeless- or Tipin-depleted NHF was accompanied by ATR- and Claspin-dependent activation of Chk1; however, co-targeting of Timeless with ATR did not restore normal BrdU incorporation, indicating a role for Timeless in DNA replication upstream of ATR. Timeless, Tipin, and Claspin contributed to sister chromatid cohesion (SCC) independently of Chk1, and Timeless had a Tipin-independent contribution to SCC. Depletion of Timeless also had a greater effect on chromosome instability than depletion of Tipin, Claspin or Chk1, possibly due to impaired cohesion in Timeless-depleted NHF. ATR-depleted NHF failed to activate the DNA damage response despite considerable chromosome instability. These results indicate that ATR, Chk1, Timeless, Tipin, and Claspin protect replication fork stability through different mechanisms in response to intrinsic challenges to DNA replication. Presently, we are characterizing a doxycycline-inducible system in NHF for structure-function studies of Timeless and Tipin.

NOTES:

P2

Analysis Of Molecular Splint Mutants Of Human DNA Pol Eta

Beardslee, R.A. and McCulloch, S.D.
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DNA polymerase eta (pol eta) is responsible for the bypass of both cyclobutane pyrimidine dimers (CPDs) and 8-oxoguanine (oxoG) during DNA replication. Both are ubiquitous; the former is produced by exposure to UV radiation, while the latter is generated by reactive oxygen species (ROS). It follows that pol eta is indispensable to successful DNA replication and organism survival. It has been reported that amino acids 316-324 appear important to correctly align the template strand with the catalytic core of the enzyme. We hypothesized that modification of these residues would interfere with that alignment and alter enzyme activity and fidelity. To study the effect of these mutations, we expressed the catalytic core of human pol eta in *E. coli* with single amino acid substitutions at residues 316, 318, 320, 322 and 324 in addition to wild type enzyme. Overexpressed protein was purified by chromatography using HiTrap™ Chelating HP (GE) with subsequent application of pol eta rich fractions to Mono S™ (GE). Purified protein fractions and DNA oligomers synthesized with both CPD and oxoG lesions were used in *in vitro* assays to evaluate polymerase properties during replication of undamaged and damaged DNA. We find that certain mutations alter the activity and/or fidelity of pol eta compared to wild type and propose that these results suggest that these amino acids are important for successful bypass of DNA lesions. Furthermore, we believe that the findings will contribute to an explanation for the low bypass fidelity of pol eta past oxoG lesions.

NOTES:

P3

Differential effects of PARP inhibition and depletion on single- and double-strand break induction/repair in human cells are revealed by changes in EBV

Wenjian Ma, Chris J. Halweg, Daniel Menendez, Michael A. Resnick
Chromosome Stability Section, National Institute of Environmental Health Sciences (NIEHS), NIH, Research Triangle Park, NC 27709

Poly ADP-ribose polymerase (PARP) inhibitors can generate synthetic lethality in cancer cells defective in homologous recombination. However, the mechanism(s) by which they affect DNA repair has not been established. Here, we directly determine the effects of PARP inhibition and PARP1 depletion on the repair of ionizing radiation-induced single- and double-strand breaks (SSBs & DSBs) in human lymphoid cell lines. This was accomplished using a newly developed *in vivo* repair assay based on large endogenous Epstein Barr virus (EBV) circular episomes. The EBV-break assay provides unique opportunities to quantitatively and simultaneously assess the induction and repair of SSBs and DSBs in human cells. Repair was efficient in both G1 and G2 cells and was not dependent on functional p53. PARP1 protein was not essential for SSB repair based on shRNA inhibition of PARP1. Among ten widely used PARP inhibitors, none affected DSB repair although a Ku inhibitor was highly effective at reducing repair of the broken EBV minichromosomes. Only Olaparib and Iniparib which are in clinical cancer therapy trials as well as 4-AN strongly inhibited SSB repair. However, a decrease in PARP1 expression reversed the ability of Iniparib to reduce SSB repair. Since Iniparib disrupts PARP1-DNA binding, the mechanism of inhibition does not appear to involve trapping of PARP at SSBs.

NOTES:

P4

Active Site Mutants of DNA Polymerase Eta Alter Fidelity and Bypass of 8-oxo-Guanine

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Y-family DNA polymerases are required to bypass lesions encountered during DNA replication, preventing replication fork collapse and DNA strand breaks. UV light can cause not only cyclobutane pyrimidine dimers (CPD), but also oxidative damage such as 7,8-dihydro-8-oxo-guanine (8-oxo-G). DNA polymerase η (pol η) is able to bypass both CPD and 8-oxo-G. To investigate the role of residues implicated in the bypass of DNA damage, we have created and purified a set of single amino acid substitution mutants of pol η. These mutants were tested to determine polymerase activity, ability to synthesize past damage, and insertion fidelity opposite undamaged and damaged DNA. Our results indicate that these residue changes can alter polymerization activity of the enzyme, as well as fidelity and bypass past DNA lesions. Some changes can confer a mutator phenotype, while others have an opposite effect. These results indicate that pol η walks a fine line between the ability to bypass lesions while still maintaining adequate replication fidelity. The increase in bypass at the cost of decreased fidelity has implications for an organism at multiple levels. The characterization of these mutants gives us insight as to the molecular determinants for the most important properties of pol η in performing its function of bypassing lesions and preventing mutation during lesion bypass.

NOTES:

P5

Prediction of Pathway Activation by Xenobiotic-Responsive Transcription Factors in the Mouse Liver

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Many drugs and environmentally-relevant chemicals activate xenobiotic-responsive transcription factors (TF). Identification of target genes of these factors would be useful in predicting pathway activation in in vitro chemical screening. Starting with a large compendium of Affymetrix files (>2000), we identified sets of signature genes that are dependent on specific TF including PPARalpha, CAR, PXR, AhR, Nrf2, FXR, and glucocorticoid receptor (GR). Target genes were identified by comparing the profiles after exposure to TF activators in wild-type and TF-null mice. In addition, we identified sets of genes associated with phenotypes that are linked to liver toxicity/cancer including inflammation and cytotoxicity. By using the sets of signature genes to query the compendium, a number of novel observations were made. 1) PPARalpha is activated in a number of nullizygous mouse models that also lead to liver toxicity and may be linked to steatosis. 2) Nrf2 is activated by a large number of conditions including environmentally-relevant chemicals, gene mutations, and bacterial infections. 3) GR is activated by a number of conditions including caloric restriction and systemic damage outside the liver. 4) Feminization of male-specific signature genes occurred after castration, in GH-defective dwarf mice, during bacterial infections and chemical exposure. Our approach allows for creation of new gene signatures associated with other pathways or phenotypes in the mouse liver that can be tested using the compendium. We are presently using the signature sets to predict pathway activation after chemical exposure in mouse primary hepatocytes. (This abstract does not represent EPA policy.)

NOTES:

P6

Dose-Response Evaluation of Micronucleus and p53 DNA Damage Response by Methyl Methanesulfonate, Etoposide, and Quercetin

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Introduction: Proliferating cells respond to chemical induced DNA damage by initiating the p53 pathway, leading to cell cycle arrest, damage repair or apoptosis. Methyl methanesulfonate (MMS), etoposide (ETP) and quercetin (QUE) have distinct mode of action of DNA damages. Methods: HT1080 human fibrosarcoma cell line was used to examine dose response for micronuclei, cell cycle cell death, p-H2AX and p53 activation after exposure to those chemicals. Results: Significant G2/M phase arrest is induced by the DNA topoisomerase II inhibitor ETP at doses > 0.02 μM, while QUE, a chemical which causes oxidative damage at high doses, induced a moderate level of S-phase arrest at lower doses (<=30 μM) and a strong G1 arrest at higher concentrations (>30 μM). MMS, an alkylating agent, induced a strong S phase arrest, but only at high doses (> 100 μM). All three chemicals dose dependently increased p-H2AX, p53 and p-p53, though much lower concentrations of ETP were required than QUE or MMS. ETP significantly increased micronuclei formation at doses similar to those required for activation of p53, however QUE and MMS only moderately induced micronuclei formation. Conclusion: ETP, QUE and MMS have distinct effects on p53 pathway activation, cell cycle arrest and micronuclei formation.

NOTES:

P7

Wiki-based Data Management to Support Systems Toxicology

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As the field of toxicology relies more heavily on systems approaches for mode of action discovery, evaluation, and modeling, the need for integrated data management is greater than ever. To meet these needs, we have developed a flexible system that assists individual or multiple research scientists with experimental planning and design, as well as for storing and publishing the data derived from the experiments. This system is designed with collaborative experiments in mind and easily allows sharing of data and tracking of changes to these data. The EPA WikiLIMS™ was constructed on the MediaWiki platform with experimental metadata stored inside the Wiki and links to raw data stored on the underlying file system. All aspects of the design are linked to bring the user a system that facilitates 1) planning and designing the whole life-cycle of the experiment, 2) backing up data with an automatic version-controlled mechanism so data cannot be lost and any modifications are fully documented, 3) organizing collaborators' contributions to the experiment into specified sections, 4) communicating with data analysts for comparing meta-analysis across multiple individual experiments, 5) tracking of samples via auto-generated Excel worksheets, and 6) constructing all necessary components for publication of experimental data via public repositories. With the EPA WikiLIMS™ data management and storage system, EPA scientists are able to save time and easily, efficiently and securely manage their experimental studies in one system. [This abstract does not necessarily reflect the views of the EPA or represent EPA endorsement of any product.]

NOTES:

P8

Sister chromatid cohesion suppresses diploid-dependent chromosome amplification and chromosome-wide loss of heterozygosity

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Aneuploidy is associated with several diseases, including cancer. Sister chromatid cohesion (SCC) prevents aneuploidy by holding sister chromatids together until anaphase to assure proper segregation. We have previously shown that SCC is also important for double-strand break repair (DSBR) by directing homologous recombination to sister chromatids instead of homologous chromosomes, which leads to loss of heterozygocity (LOH). We asked whether defects in SCC could also lead to aneuploidy since inefficient homologous recombination could be a source of this kind of genetic instability. Here we show that diploid (*MATa/α*) SCC mutants but not WT have a 10-fold increase in the rate of chromosome gain (CG) over isogenic haploids. Unlike in WT, DNA damage in the SCC mutants results in a synergistic increase in aneuploidy rate. Surprisingly, the rates of CG in WT and SCC mutants were increased 100-fold in a homozygous *MATa/a* strain (which has reduced DSBR) as compared to a *MATa* haploid counterpart. Thus, the CG rates of a diploid cohesin mutant are 15,000-fold higher than a haploid WT strain. SCC mutants show disproportional increase in half cross-overs, indicating a defect in resolution of recombination intermediates. We propose that SCC is needed to prevent diploid-dependent aneuploidy by suppressing interactions between homologous chromosomes.

NOTES:

P9

UV-Induced Mutagenesis at the *hprt* Locus is Not Enhanced Following Knockdown of Intra-S Phase Checkpoint Proteins ATR or Chk1

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Mammalian cells have acquired multiple mechanisms to respond to genotoxic insults, such as DNA damage caused by ultraviolet radiation (UVR). These responses include DNA repair pathways and those that allow cells to tolerate potentially mutagenic DNA template lesions. It is widely accepted that the intra-S checkpoint responds to UV-induced DNA damage to slow S-phase progression, allowing repair mechanisms to remove DNA lesions prior to replication. The intra-S checkpoint is activated in response to UVR in a manner that is dependent upon the activity of the protein kinases ATR and Chk1 at sites of stalled replication. We designed experiments to test the hypothesis that the intra-S checkpoint is protective against UV-induced mutagenesis. UV-induced mutation frequency at the *hprt* locus was assessed in human dermal fibroblasts after siRNA knockdown of either ATR or Chk1. Briefly, cells were electroporated with siRNA targeting Chk1 or ATR; following knockdown, cells were irradiated with UV and allowed to expand through 4-6 population doublings in order to fix mutations. Cells were then selected in medium containing 6-thioguanine, allowing only cells lacking functional *hprt* to form colonies. Colony yield was then used to calculate mutation frequency at the *hprt* locus. Our results showed that knockdown of Chk1 or ATR, proteins critical for intra-S checkpoint signal transduction, did not increase mutation frequency. Although further experiments are needed, these results seem to suggest that inhibition of the intra-S phase checkpoint does not enhance UV-induced mutagenesis, unless depletion of these checkpoint kinases is compromising mutation acquisition or survival of mutant cells.

NOTES:

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- Student and post-doctoral trainee GEMS members from government, colleges and businesses in North Carolina are provided an opportunity to hone their presentation skills, meet with senior investigators and peers, and learn more about new trends in biomedical research while competing for juried awards.

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