Dec 3 (3-4pm) NEXT MEETING

GEMS

Genetics and Environmental Mutagenesis Society

31st Annual Fall Meeting

"Exploiting the DNA damage response to prevent and cure cancer"

Thursday, November 14, 2013

Sheraton Imperial Hotel and Convention Center
4700 Emperor Blvd.
Durham NC 27703

Supported in part by grants from NIEHS.

National Institute of
Environmental Health Sciences

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November 14, 2013

Dear GEMS Members,

Welcome to the 31 Annual Meeting of GEMS! It seems like yesterday when Tom Barfknecht (now in Houston, Texas at Momentive Chemicals) and I were at RTI in 1982 where we came up with the proposed GEMS name before we were even organized. Now here we are many years later and after many years of hard work by many BOD members. What an achievement this Society has been. Many of the junior members back in 1982 are now senior scientists in our laboratories! Over these 31 years, GEMS has sent many scientists to national meetings and given hundreds of scientists the opportunity to present their data at our fall meetings. We have heard many great talks and seen many great posters and enjoyed many great conversations over the years.

We now have had both a spring meeting and a fall meeting for many years. David DeMarini did the first spring meeting on indoor air in 1992. The membership is somewhat smaller now but we still have an active BOD and enthusiastic members. We have a Web page actively managed by Bill Ward. We do our meeting registration electronically. We had 50 attendees at the Spring Meeting in May 2013 and we expect 60 attendees here today at the Sheraton Imperial. Bill Kaufmann has done an excellent job on organizing this meeting and please thank him and all the BOD members when you see them today.

I have many people to thank as the 2013 President. I had a very excellent BOD who worked very hard in difficult financial times. Bill Kaufmann organized two excellent scientific meetings. Cheryl Hobbs organized the elections. Carol Swartz was the Corporate Sponsor Coordinator. Bill Ward was the Web Master. George Goodall coordinated the electronic distribution of the Spring Meeting. Mark Miller supplied the coffee for the Spring Meeting. Brian Chorley did the minutes while our very able secretary, Holly Mortensen, had her second child. Rose Anne McGee was our very accurate treasurer. Barbara Shane, Jack Bishop and Sarah Warren all provided support as BOD members in decisions that affected the Society. I thank them all. We met every month, had a good time and were effective in 2013.

Please support and visit the corporate booths at our meeting today. They support GEMS and we must support them.

Finally, thank you all so much for all the great years of the GEMS. I have truly enjoyed every minute. I look forward to many more GEMS meetings in the future.

Enjoy the GEMS Meeting today.

Sincerely,

Tom Hughes, 2013 GEMS President

President-Elect's Message

Dear GEMS Members, Postdocs, Students and Guests,

With great pleasure, I welcome you to an exciting 31st Annual Fall Meeting of the Genetics and Environmental Mutagenesis Society. The theme of this meeting is **"Exploiting the DNA damage response to prevent and cure cancer"**.

The field of DNA damage responses has expanded considerably over the past 40 years motivated first by the demonstration that many carcinogens were mutagens that damage DNA, second by the identification of familial cancer syndromes with defects in DNA repair and third by the realization that many tumor suppressor genes are components of the DNA damage response. The Loeb mutator theory of carcinogenesis holds that the normal rate of mutation is too low to generate the 6-8 independent mutations needed to produce cancer. As cancer is a common disease, an early event in carcinogenesis must increase the rate of mutation. It is very likely that germline and somatic mutations in *TP53, HMLH1* and *ATM* are mutators that increase the rate of mutation. **Dr. Michael Kastan** of Duke University will tell about his work to translate his discoveries on *TP53* and *ATM* to more effective treatments for cancer.

Pathways of DNA repair and DNA damage checkpoints protect against development of cancer by reducing the levels of DNA damage or enhancing the time available for repair of the damage. These DNA damage responses not only protect against the development of cancer they protect cancer cells from radiation and chemotherapies that seek to cure the disease. The demonstration of synthetic lethalities where a weakly toxic insult can be transformed into a highly toxic lesion by modification of gene expression has renewed interest in the DNA damage response. Inhibitors of poly(ADP-ribose) polymerase have modest toxicity normally but in cells with inactivation of BRCA1-dependent homologous recombination these drugs have massive toxicity. We seek now development of new combinations of drugs to kill cancer cells with greater specificity and thereby enhance cure rates. **Dr. William Gmeiner** of Wake Forest University will describe his efforts to target DNA topoisomerase I for more effective treatment of leukemia and lymphoma.

A remarkable discovery was the demonstration that the XPA nucleotide excision repair factor varied in its expression according to the time of day. Circadian regulation of NER in skin implies that our risk of skin cancer may vary with the time of harmful UV exposure. Circadian regulation of NER may also influence the efficacy of chemotherapies and this implies that the timing of treatment (and standard of care) may need to be modified. **Dr. Aziz Sancar** of the University of North Carolina will report on his efforts to define the role of the circadian clock in environmental carcinogenesis and how clock mechanisms may be exploited to prevent or cure cancer.

I look forward to seeing you all at the meeting.

William Kaufmann, GEMS President-elect

GEMS

Genetics and Environmental Mutagenesis Society

Exploiting the DNA damage response to prevent and cure cancer

8:00-8:45 am Arrival and registration with continental breakfast

8:45 am	Welcome	William Kaufmann
9:00 - 9:45 am	"DNA damage responses: bedside to bench to bedside" Michael Kastan	
9:45 - 10:30 am	"Potentiating Top1 Poisons by Modulating the DNA Damage Response – New Strategies for AML Treatment" William Gmeiner	
10:30- 11:00 am	Coffee break	
11:00- 11:15 am	"Biochemical Analysis of DNA Polymerase η Fidelity in the Presence of Replication Protein A". Samuel C. Suarez, NCSU	
11:15-11:30 am	"Arsenite and methyl methanesulfonate (MMS) co-exposures induce synergistic cellular responses associated with carcinogenic pathways" Pergentino Balbuena, The Hamner Institutes of Health Sciences	
11:30-11:45 am	"Functional genomics approach in yeast identifies DNA repair genes important in response to trichloroethylene" Vanessa DeLaRosa, UNC-CH/UC-Berkeley	
11:45-12:00 pm	"C/EBPα regulates $p21^{CIP1/WAF1}$ protein levels during the UVB-induced DNA damage checkpoint response" Jonathan R. Hall, NCSU	
12:00-1:15 pm	Lunch	
1:15-2:45 pm	Poster viewing with corporate sponsors	
2:45-3:30 pm	"Control of DNA Repair and Cancer by the Circadian Clock" Aziz Sancar	
3:30 pm	GEMS business meeting and awards presentations Tom Hughes	
4:00 pm	Adjourn	

Mice excision repair
High replication + low repair in mice in AM
↓ rep + ↑ repair in PM

Circadian rhythm in vitro? can you establish?

Aziz Sancar, M.D. University of Istanbul, Ph.D. University of Texas

Distinguished Professor of Biochemistry and Biophysics, University of North Carolina at Chapel Hill

RESEARCH INTERESTS: http://www.med.unc.edu/biochem/asancar

DNA Repair

We are studying the molecular mechanism of nucleotide excision repair in humans. This is a general DNA repair system that repairs all base lesions including the carcinogenic lesions induced by the main environmental carcinogens sunlight and cigarette smoke. Our lab was the first to reconstitute the excision nuclease in a defined system. Our current work on excision repair aims to understand the structural and kinetic factors that enable the human excision nuclease to remove virtually infinite types of base lesions and to define the interconnections between DNA excision repair, the DNA damage checkpoints, and the circadian clock.

DNA Damage Checkpoints

DNA damage checkpoints are biochemical pathways that transiently block cell cycle progression while the DNA contains damage. Checkpoints prevent genomic instability, cancer, and death in multicellular organisms. The DNA damage checkpoints, like other signal transduction pathways, have four components: damage sensors, mediators, signal transducers and effectors. The goal of our research is to purify the human checkpoint proteins, characterize these proteins biochemically, and reconstitute the DNA damage checkpoint in vitro. We have already established an in vitro system that recapitulates some of the key features of the human DNA damage checkpoint response to base damage. Abnormal checkpoint response to DNA damage is a universal feature of cancers, and biochemical characterization of the checkpoint response should aid in developing new approaches to cancer chemotherapy.

Cryptochrome and Regulation of the Biological Clock

Circadian rhythm is the oscillation in physiology and behavior of organisms with approximately 24-hour periodicity. The circadian clock is synchronized to the daily solar cycle by light. We have discovered that a flavoprotein called cryptochrome, closely related to the light-dependent DNA repair enzyme photolyase, regulates the mammalian circadian clock by light-independent and light-dependent mechanisms. Currently, we are investigating the action mechanism of cryptochrome using biophysical methods including femtochemistry and biochemical methods. In addition, we are investigating the connection between the circadian cycle and DNA repair and how disruption of the circadian cycle might affect the susceptibility of mice and humans to cancers.

Michael B. Kastan, M.D., Ph.D., Washington University

Executive Director, Duke Cancer Institute
Professor of Pharmacology and Cancer Biology
Professor of Pediatrics, Duke University

- ↑p53 translation via RPL26 Chen + Kastan, 2010 (Genes+Dev)
- DS structure of p53 coding sequence where RPL26 binds ∴ ↑ translation
- oligonucleotide that blocks site inhibits p53 for most exposure except nothin -
- helps prevent in vitro "culture shock"

Research Interests: http://pharmacology.mc.duke.edu/faculty/kastan.html

Cellular responses to DNA damage and other stresses are important determinants of cell viability and mutagenesis and impact the development of a wide range of human diseases. The ability to modulate cellular responses to DNA damage and other stresses can impact cancer development, tumor responses to therapy, the organ toxicities of cancer treatments or accidental exposure to radiation or other DNA damaging agents, the development of cardiovascular disease, outcome (extent of organ damage) following heart attack or stroke, and the rate of progression of certain neurodegenerative disorders. The focus of the Kastan lab has long been related to elucidating molecular mechanisms involved in cellular responses to DNA damage and other stresses. This began with studies of changes in DNA methylation and chromatin structure/modification during the process of DNA excision repair (Cell, 1982) and continued with a series of major discoveries demonstrating that the p53 protein, the most commonly mutated gene in human cancer, plays a critical role in enabling mammalian cells to cope with DNA damage, in particular by controlling progression of cells from the G1 phase of the cell cycle into S-phase (Cancer Research, 1991; PNAS, 1992; Cell, 1992) and regulating apoptosis. In the subsequent 20 years since the discovery of this role for p53, the lab has continued efforts to elucidate molecular mechanisms involved in DNA damage, including cell cycle control, programmed cell death signaling, DNA repair mechanisms, DNA damage-induced signal transduction pathways, and more recently interfaces between cellular metabolism and stress signaling. This has included generating many seminal insights into the roles and mechanisms of action of the ATM protein kinase, including its role in signaling to p53 (Cell, 1992; Genes and Development, 1997; Science, 1998), identifying other ATM substrates involved in DNA damage signaling (Nature, 2000; Genes and Development, 2002, 2004), and elucidating the mechanism of ATM activation (Nature, 2003). In the past several years, the lab has identified a novel and unexpected mechanism involved in the stress-induction of p53, namely a stimulation of the translation of p53 mRNA and recent insights have been gained into the molecular controls of this translational increase (Cell, 2005; Molecular Cell, 2008; Genes and Development, 2010) that are leading to development of small molecules that have the potential to modulate p53 induction after stress and protect normal tissues from radiation, chemotherapy, or hypoxia-reperfusion injury. Recent ATM studies in the lab have focused on molecular controls of DNA double strand break repair (Nature Cell Biology, 2007), its role in insulin signaling, metabolic syndrome, and mitochondrial function (Nature Cell Biology, 2000; Cell Metabolism, 2006; unpublished studies), and the development of small molecule inhibitors to be used as clinical tumor radiosensitizers (Cancer Research, 2008; unpublished studies).

- γ-H2AX + comet assay: unsatisfactory methods to measure DNA breaks + repair
- another method in PNAS 2013, using ChIP + PCR: w/ 1 Gy rad., see damage @ 1h, then repair @ 24
- Nucleotide [illegible] recruitment (XRCC4, for ex.)

William H. Gmeiner, Ph.D., University of Utah

Professor of Physiology and Pharmacology, Cancer Biology Comprehensive Cancer Center, Brain Tumor Center of Excellence, Wake Forest School of Medicine

Research interests: http://www.wakehealth.edu/faculty/Gmeiner-William-Henry.htm

My laboratory is interested in understanding how efficacious anti-cancer drugs cause cancer cell death and in designing new drugs and novel drug delivery strategies. Fluoropyrimidines (FPs) are an example of a class of drugs that is widely used for cancer chemotherapy, yet the cytotoxic mechanisms of FPs are not completely understood and clinical response to these drugs is limited. I invented a novel polymeric form of FdUMP, the TS-inhibitory metabolite of 5FU, and have demonstrated the safety and efficacy of the FdUMP[10] polymer in tissue culture and animal models of human cancer. The results indicate that FdUMP[N] polymers are likely to be highly efficacious for the treatment of human cancer. Further, FdUMP[N] polymers are highly cytotoxic towards cancer cells that are not sensitive to 5FU, such as prostate cancer cells, but are not cytotoxic to normal prostatic epithelial cells.

The increased activity of FdUMP[N] polymers relative to monomeric FPs results from the increased misincorporation of FdUTP into DNA and greater DNA damage. In collaboration with Dr. Yves Pommier (NCI), we showed that exposure of cancer cells to FdUMP[10] results in trapping of topoisomerase I (Top1) cleavage complexes at the site of FdUTP misincoporation. Thus, FdUMP[10] mechanistically resembles the camptothecin class of anticancer drugs. [ATP-levels in drug-treated cells are assessed using a luminescence assay]. In collaboration with Dr. Frits Peters (Free University, Amsterdam) we recently showed that FdUMP[10] retained activity towards TK-null cells while FdU does not implicating cellular degradation to FdU is not essential for FdUMP[10] cytotoxicity. Current efforts in my laboratory are focused on elucidating the cell death pathways that are activated in human prostate cancer cells following exposure to FdUMP[10]. My laboratory has also demonstrated that FdUMP[10] is a potent radiosensitizer of prostate cancer xenografts in vivo.

A major new initiative in my laboratory in recent years is the development of highly structured DNA molecules that are selectively cytotoxic to cancer cells. The selective killing of malignant cells remains an important challenge that has not been fully met by the development of monoclonal antibodies and other targeted therapeutics. The "Cytotoxamers™" being developed in my laboratory represent a novel approach to the selective killing of cancer cells. These structured molecules are less prone to extracellular degradation than linear homopolymers and have the potential to be highly selective anticancer drugs ushering the antimetabolite class of anticancer drugs into the era of targeted therapeutics.

5FU → highly neurotoxic in dogs + cats
↳ there [illegible] is metabolite that is toxic

- Top1CC activates NFKB + c-Jun modulating the survival/apoptosis balance
(Oncogene (2012) 31, 537-551) ATM dependent process?

T1

Biochemical Analysis of DNA Polymerase η Fidelity in the Presence of Replication Protein A

Samuel C. Suarez and Scott D. McCulloch
North Carolina State University, Raleigh, NC

DNA polymerase η (pol η) synthesizes across from damaged DNA templates in order to prevent deleterious consequences like replication fork collapse and double-strand breaks. This process, termed translesion synthesis (TLS), is an overall positive for the cell, as cells deficient in pol η display higher mutation rates. This outcome results despite the fact that the *in vitro* fidelity of bypass by pol η alone is moderate to low, depending on the damage being copied. One of the means proposed to raise the fidelity of pol η is interaction with replication accessory proteins present at the replication fork. We have previously utilized a phage-based color screening system to measure the fidelity of bypass using purified proteins. Our analysis has focused on the fidelity effects of single stranded binding protein, replication protein A (RPA), when copying undamaged DNA, and 8-oxoG. We observed no effect of RPA when copying these templates. This result is consistent in multiple position contexts, as well as when copying TT dimer. We previously identified single amino acid substitution mutants in a truncated form of pol η that have specific effects on fidelity when copying all 3 templates. In order to confirm our results, we examined the Q38A and Y52E mutants in the same full length construct. We again observed no difference when RPA was added to the bypass reaction, and the mutants displayed similar fidelity to their truncated counterparts. Our results indicate that the binding of RPA to DNA does not affect pol η bypass fidelity.

T2

ARSENITE AND METHYL METHANESULFONATE (MMS) CO-EXPOSURES INDUCE SYNERGISTIC CELLULAR RESPONSES ASSOCIATED WITH CARCINOGENIC PATHWAYS

Pergentino Balbuena[1], Susan M. Ross[1], Les Recio[2], John Winters[2] Harvey J. Clewell[1], Rebecca Clewell[1]
[1]The Hamner Institutes of Health Sciences, [2]Integrated Laboratory Systems

Arsenite inhibits DNA repair, exacerbating effects of genotoxic chemicals. MMS is an alkylating agent that induces DNA strand breaks. The purpose of this study was to determine how concurrent exposure to arsenite alters cellular responses to DNA damage from a direct mutagenic compound s *in vitro*. We assessed genotoxicity (micronucleus formation), DNA damage (Comet assay, pH2AX protein), and proteins associated with apoptosis (Bcl2), antioxidant response (NQO1), and DNA damage response (p53) following 24 hr treatments with arsenite and MMS. Arsenite at 3 nM and 300 nM combined with MMS at 1, 3, 10, 30, and 100 µM were assessed in the HT-1080 human fibrosarcoma cell line (expresses wild-type p53). Cells showed similar NQO1 and p53 responses to arsenite compared to primary cell cultures of human uroepithelial cells (target for arsenic-induced bladder cancer), indicating that the HT1080 is a reasonable *in vitro* model for arsenite response. 3nM Arsenite/MMS mixtures induced significant increases in protein expression for Bcl2 and NQO1 when compared to MMS alone. Co-exposure to 300 nM arsenite increased the expression of these proteins at least two fold compared to MMS alone. Co-exposures did not significantly change the p53 response. Mutagenic effects were not detected by micronucleus analysis, even at the highest concentrations; however, increased DNA damage was detected by the comet assay and pH2AX expression in the mixtures. DNA damage and apoptosis markers showed increases consistent with a synergistic response. These results suggest that the co-mutagenicity of arsenite involves increased survival signaling, together with inhibition p53 DNA damage response.

NOTES:

- Using primary human bladder cell model (HUECs)
  → maintained characteristics for 3 passages
- HT-1080, alternative immortal cell line (fibrosarcoma)
- As exposure + looked @ NQO1 response in both cell-types

* exposure that ↑ OS, DNA mutation, ↓ apoptosis / no p53 response ; ↑ NQO1 response (NRF2?)

~ used combination of DSB (MMS treatment) + O.S. exposure (As)

T3

Functional Genomics Approach in Yeast Identifies DNA Repair Genes Important in Response to Trichloroethylene. V. De La Rosa, J. Asfaha, C. Vulpe, University of California, Berkeley, CA.

Trichloroethylene (TCE) is an industrial solvent and common environmental contaminant, particularly at Superfund waste sites. TCE is deemed a human carcinogen, yet the molecular events mediating toxicity and cancer remain convoluted. We aim to utilize a functional genomics approach in model organisms to gain insight on the genes that modulate TCE toxicity. Initial studies utilized parallel deletion analysis in yeast to identify genes important in the response to TCE exposure. This approach identified a specific subset of DNA repair genes, suggesting a role for the DNA repair response in mediating toxicity in yeast. Subsequent functional studies were conducted in transformed avian lymphocyte cell lines (DT40) to assess whether the DNA repair response was conserved.

Loss of translesion synthesis and NER genes in both yeast and DT40 cells resulted in a significant decrease in viability upon exposure to low levels of TCE, but sensitivity was not seen in these mutants at higher doses. Additional analyses in DT40 cells revealed elevated levels of double strand breaks at high doses of TCE. These results suggest the DNA repair response to TCE is dose dependent, resulting in the potential for mutagenesis. Studies assessing mutagenesis using the TK6 mammalian cell line are ongoing. In conclusion, these studies have identified cellular targets that may play significant roles in mediating TCE toxicity.

NOTES:

DT40 cell panel: lymphoblast cell lines w/ deficiency in repair enzymes

T4

C/EBPα regulates p21$^{CIP1/WAF1}$ protein levels during the UVB-induced DNA damage checkpoint response

Jonathan R. Hall, Elizabeth A. Thompson, and Robert C. Smart.

Cell Signaling and Cancer Group, Department of Biological Sciences, Center for Human Health and the Environment, North Carolina State University, Raleigh, North Carolina
Nonmelanoma skin cancer (NMSC) is the most common cancer in the United States; each year there are more cases of NMSC than all cases of breast, prostate, lung and colon cancers combined. The majority of NMSCs are caused by solar UVB radiation. Keratinocytes respond to UVB-induced DNA damage by engaging the DNA damage response which involves cell cycle arrest, DNA repair and apoptosis. Defects in any one of these responses can lead to mutations and cancer. Mice containing a skin specific ablation of C/EBPα are highly susceptible to skin cancer development following chronic low doses of UVB radiation. Moreover, C/EBPα-deficient keratinocytes in culture or in vivo in mouse epidermis fail to undergo cell cycle arrest in G1 in response to UVB-induced DNA damage, thus allowing cells with damaged DNA to inappropriately enter S-phase. Despite these critical and novel roles for C/EBPα in prevention of UVB-induced skin cancer and the cellular response to DNA damage involving cell cycle arrest, the molecular mechanisms and key players involved downstream of C/EBPα in the DNA damage G_1 checkpoint response remain uncharacterized. We have recently discovered that C/EBPα plays an essential role in the UVB-induced accumulation of the cyclin dependent kinase (CDK) inhibitor p21$^{CIP1/WAF1}$. P21 is highly induced by DNA damage and is a potent inhibitor of CDK2, a critical target in the G_1 DNA damage checkpoint response. We propose that the C/EBPα-mediated accumulation of p21 in response to UVB radiation is essential for a proper G_1 DNA damage response and the prevention of NMSC.

NOTES:

→ always nuclear + bound to chromatin
C/EBPα → possible tumor suppressor? via cell cycle arrest mediation (cellular differentiative phenotype)

p53→ p21 inhibits cyclin-CDK2 complex ⊣ G1/S transition

C/EBPα regulates p21 post-translationally? ∴ mediates cell cycle arrest

∴ C/EBPα serves as a mediator of p21-mediated cell cycle arrest post-transcriptionally

silencing C/EBPα reduces p21 half-life which is mediated by proteasome degradation

Is p21 protective or pro-apoptotic?

Poster abstracts

P1

Nanoparticle Mediated Thermal Therapy for Glioblastoma

Brittany Eldridge, Brian Bernish and Ravi Singh
Wake Forest University Health Sciences, Winston Salem, NC

No current treatment of glioblastoma is curative. Cytoreductive treatment is needed for radiation and chemotherapy to have even limited efficacy, but surgery is not an option for most patients. Heat delivered by interstitial lasers is now being used as a focal cytoreductive treatment. Current laser-based therapies are ineffective for treatment of tumors larger than 2 cm due to inefficient heat delivery. We are studying methods to increase the efficacy of laser-based treatment using a technique called Carbon Nanotube Mediated Thermal Therapy (CNMTT). CNMTT uses laser energy to excite multiwalled carbon nanotubes introduced to the tumor site to generate highly localized hyperthermia and greatly increase the delivery of heat to the tumor. Because of diffuse infiltration of cancer cells into the surrounding normal brain tissue, complete elimination of glioblastoma cells is not achievable by the use of focal treatment modalities, and cells exposed to sub-lethal heat stress could develop resistance to subsequent treatment. We used a forced resistance model where U87 glioblastoma cells were repeatedly exposed to sub-ablative temperatures, either using conventional heating by water-bath or rapid heating through CNMTT. Our studies indicate that cells previously exposed to conventional heat treatment exhibited increased tolerance compared to control cells when subsequently exposed to sub-ablative temperatures. In contrast, U87 cells do not develop tolerance to heating through CNMTT following prior exposure to sub-ablative temperatures induced by CNMTT. Further studies are needed to better understand the mechanism of action of CNMTT and the effects of CNMTT on cancer cell survival and death pathways.

NOTES:

P2

Silver Nanoparticles Sensitize Breast Cancer Cells to Radiation

Jessica Swanner and Ravi Singh
Wake Forest University Health Sciences, Winston Salem, NC

We are studying the use of silver nanoparticles (AgNPs) as radiation sensitizers for the treatment of breast cancer (BC). If lower doses of ionizing radiation (IR) can be utilized for therapy, the patient will experience fewer side effects while attaining a superior outcome due to our therapeutic regimen. Initially, we treated both tumorigenic and non-tumorigenic breast cells with increasing doses of AgNPs (0-100 µg/ml) for 24hrs. Malignant breast cell lines (MCF-7 and triple negative MDA-MB-231 cells) were significantly more sensitive to AgNPs than non-malignant MCF-10A breast cells as indicated by MTT, BrdU, and clonogenic assays. Subsequently, all three cell lines were pre-treated with AgNPs then exposed to IR (0-4 Gy). AgNP treatment plus IR additively increased the number of DNA double strand breaks (DSBs) in all three cell lines as indicated by phospho-H2AX ELISA. MCF-7 and MDA-MB-231 cells were unable to repair DSBs at 22 hrs post treatment, while MCF-10A cells quickly repaired the damage. Mechanistically, AgNPs did not appear to inhibit DNA DSB repair, but there was evidence that AgNPs depleted glutathione and caused oxidative damage which was visualized by fluorescence microscopy. Clonogenic assays indicated that AgNPs exerted a synergistic cytotoxic effect on BC cells when combined with IR suggesting that AgNPs may be a useful adjuvant in the treatment of BC. AgNPs showed minimal toxicity to kidney cells, liver cells, and macrophages. In conclusion, AgNPs sensitize cancer cells to IR via glutathione depletion while causing minimal damage to non-tumorigenic breast cells and cells derived from blood filtering organs.

NOTES:

P3

Detrimental effects of UV-B radiation in an XP-Variant cell line

Kimberly N. Herman, Shannon Toffton, Scott D. McCulloch
Department of Biological Sciences, Environmental and Molecular Toxicology Program, North Carolina State University

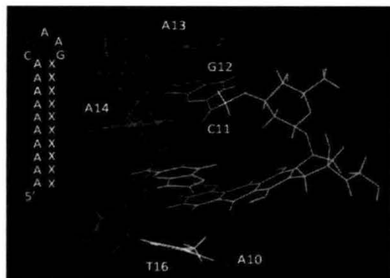
DNA polymerase η (pol η), of the Y-family, is well known for its *in vitro* DNA lesion bypass ability. The most characterized bypass event for this polymerase is that of the cyclobutane pyrimidine dimers (CPDs) caused by ultraviolet light. Historically, cellular and whole animal models for this area of research have been conducted using UV-C (254-280 nm) due to its ability to generate large quantities of CPDs. While UV-C is useful as a laboratory tool, exposure to it generally is very low due to being filtered by stratospheric ozone. Instead, we are interested in the more environmentally relevant wavelength range of UV-B (280-315 nm) for its role in causing cytotoxicity and mutagenesis. We evaluated these endpoints in both a normal human fibroblast control line and a xeroderma pigmentosum variant (XP-V) cell line; in which the POLH gene contains a truncating point mutation leading to a non-functional polymerase. We demonstrate that UV-B has similar but less striking effects compared to UV-C in both its cytotoxic and mutagenic effects. Analysis of the mutation spectrum after a single dose of UV-B shows a majority of mutations can be attributed to bypass of CPDs. However, we note additional types of mutations with UV-B that are not previously reported after UV-C exposure. We speculate these differences could be attributed to either a change in the spectrum of photoproduct lesions, or by additional lesions generated, possibly by oxidative stress.

NOTES:

P4

DNA Hairpins for Targeted Drug Delivery

Christopher Stuart, William H. Gmeiner
Wake Forest University School of Medicine, Winston-Salem, NC



Chemotherapeutic drugs lack the ability to selectively target malignant cells, giving rise to the myriad of side effects associated with cancer treatment. In order to alleviate these symptoms, delivery vehicles have been evaluated for their ability to increase a drug's therapeutic index. We demonstrate that DNA hairpins can be used as a novel drug delivery system. The use of DNA-hairpins as delivery vehicles facilitates the targeting of multiple drugs to malignant cells. Hairpins are comprised of a "GAA" loop closed with a GC base pair followed by 10 AT or AF base pairs, where F is the cytotoxic thymidine analogue, 5-fluro 2'-deoxyuridine 5'-O-monophosphate. Hairpins were covalently bound to Doxorubicin and used for cytotoxicity and stability studies. N15 labeled guanine was incorporated into hairpins for HSQC NMR to determine Dox binding sites. Hairpins were also targeted with either biotin or folate. DNA-Drug complexes displayed similar cytotoxicity as parent drugs, demonstrating that drug activity is not reduced when loaded into the hairpin. The stability of the Dox-hairpin bond was found to be approximately 30 hours, with G12 of the hairpin being the exclusive site of reactivity. Without the covalent bond to the hairpin Dox rapidly intercalated into salmon sperm DNA, suggesting that if DNA is used to deliver Dox, covalent attachment maybe necessary. Targeting hairpins with folate increased both cellular uptake and cytotoxicity of DNA-drug complexes. DNA-hairpins are a new type of drug delivery vehicle that are capable of targeting multiple drugs and may serve to reduce side-effects *in vivo*.

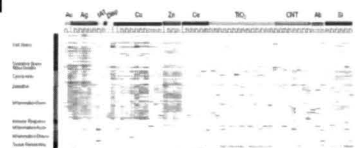
NOTES:

P5

Comparing bioactivity profiles of diverse nanomaterials based on high-throughput screening (HTS) in ToxCast

Amy Wang, US Environmental Protection Agency (US EPA), RTP, NC

Testing the fast growing numbers of nanomaterials (NMs), most lacking hazard data, requires efficient toxicity tests. The EPA's ToxCast program uses HTS assays to prioritize NMs for targeted testing and identifying biological pathways affected. Nanoparticles [Au, Ag, CeO_2, $Cu(O_2)$, TiO_2, SiO_2, ZnO core], their ion and micro counterparts, carbon nanotubes (CNTs), and asbestos were screened for 262 bioactivity/toxicity endpoints in various cells and zebrafish embryos, ranging from transcription factor activation to phenotypic changes. Pathways affected were primarily cellular stress and immune response. Core chemical composition was more important than size for bioactivity. NMs had similar profiles as their ion counterparts, suggesting ion shedding was a key factor in mechanism of action. Ag, Cu, and Zn (nano, ion) were more cytotoxic and active than others. Microparticles, except micro-ZnO, were much less active than their nano or ion counterparts. Furthermore, while 3 asbestos samples had similar immune response profiles, 6 CNTs had profiles distinctive from each other, and from asbestos. Comparing our data to reference profiles in primary human cells suggested targets that weren't directly measured, e.g. similar profiles of nano-TiO_2 and a microtubule stabilizer interfering with mitosis, a previously reported effect. Dividing endpoints into cytotoxicity and various functional/pathway groups, we developed a ToxPi-based prioritization approach. Highest priorities for follow-up (targeted) testing were some of the Ag, Cu, and Zn samples. Lower priority samples included asbestos, Au, CeO_2, some CNTs, and some TiO_2 samples. We demonstrated that HTS assays can identify cellular pathways, predict targets, and prioritize NMs for future research.



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P6

Ablation of the bZIP transcription factor, CCAAT enhancer binding protein-β (C/EBPβ), in skin protects mice from skin cancer induced by ultraviolet B radiation

H.W. Tam, S. Zhu and R.C. Smart
Department of Biological Sciences, Toxicology Program, NC State University, Raleigh, NC

Nonmelanoma skin cancer (NMSC) is the most common cancer in the United States. The majority of NMSCs are caused by solar UVB radiation which causes DNA damage. We observed that C/EBPβ is abundantly expressed in epidermis and that C/EBPβ protein levels are induced by UVB radiation in mouse and human keratinocytes in culture and in mouse epidermis *in vivo*. UVB treatment resulted in the increased phosphorylation of C/EBPβ at threonine-188, which is an activated form of C/EBPβ. In order to understand the function of C/EBPβ in UVB-induced responses in skin cancer, we developed a C/EBPβ deficient (C/EBPβ$^{-/-}$) SKH-1 hairless mouse model. SKH-1 hairless mice are an experimental model relevant to UVB-induced human skin cancer. Treatment of C/EBPβ$^{-/-}$ mice with UVB, resulted in increased apoptotic cell death and increased p53 levels in epidermal keratinocytes when compared to control mice. C/EBPβ$^{-/-}$ mice were highly resistant to UVB-induced skin cancer. In order to determine whether the resistance of C/EBPβ$^{-/-}$ mice to UVB-induced skin cancer is due to an intrinsic keratinocyte effect, we developed an epidermal specific C/EBPβ conditional knockout (CKOβ) SKH-1 mouse. In this model, the keratin 5 promoter directs Cre recombinase expression to the epidermis to delete floxed C/EBPβ alleles. CKOβ mice were highly resistant to UVB-induced skin cancer and displayed increased levels of UVB-induced apoptosis. These studies indicate that C/EBPβ is induced and activated by UVB and has critical role in the development of UVB-induced skin cancer where it suppresses UVB-induced apoptosis and in doing so may allow the survival of keratinocytes with DNA damage and mutations.

Notes:

P7

Genome architecture and dynamics drive mutagenesis via replication infidelity.

Scott Lujan, Anders Clausen, Dmitry Gordenin, Alan Clark, Jason Belsky, David MacAlpine, Adam B. Burkholder, Piotr Mieczkowski, David Fargo, Michael Resnick, Thomas Kunkel.
National Institute of Environmental Health Sciences, NIH, DHHS, Research Triangle Park, NC 27709
An enduring question in DNA replication research is why some sequences are much more susceptible to mutations than others. Mutation patterns in both the evolutionary record and cancer have been attributed to biased DNA damage, damage repair, polymerase fidelity, mismatch repair (MMR), and purifying selection. We dissect these hypotheses with whole genome mutation accumulation experiments in diploid *Saccharomyces cerevisiae*. After up to 900 generations of divergence, we found over 40,000 mutations in 54 isolates of eight strains that varied by MMR proficiency and polymerase mutator status. We found that there are general motifs for replication errors and deciphered mechanistic implications thereof. Replication errors and MMR are strand- and polymerase-dependent and canonical division of polymerase labour across the yeast genome. We also found that MMR results in a correlation between variation and replication timing, as seen in evolutionary history and in cancer. Other evolutionary mutation patterns are explained by replication fidelity biases. Where selective pressures are weak, as in around nucleosomes and 3'-UTRs, replication errors recapitulate past variation. Where selection is strong, as in genes and promoters, replication errors are anti-correlated with evolutionary sequence conservation. Thus, in the absence of selection and MMR, we reveal the fingerprints of past selective pressure and future mutational vulnerability relevant to evolution and possibly disease aetiology.

NOTES:

P8

Oncogenic BRAF Induces a UV-Mutator in Melanoma Cells

Dennis Simpson, Nathaly Lemonie, David Morgan, HaeYon Lee, William Kaufmann
University of North Carolina at Chapel Hill

Cell cycle checkpoints are surveillance systems that curb cell division when conditions are inopportune or inappropriate. It is inopportune to initiate DNA synthesis when DNA is damaged and inappropriate to begin mitosis before DNA replication is complete. Defects in checkpoint function enhance cell growth and genomic instability fueling malignant progression. We have quantified DNA damage checkpoint function in normal human melanocytes and melanoma cell lines to ask whether defects in checkpoint function accompany melanomagenesis. We present evidence demonstrating that an initiating event in melanomagenesis, mutational activation of the melanoma oncogene *BRAF*, attenuates DNA damage G2 checkpoint function and sensitizes melanoma cells to ultraviolet radiation-induced DNA damage. In melanoma cells with wildtype *BRAF* and effective G2 checkpoint function, induction of oncogenic BRAF (V600E) attenuated the G2 checkpoint and increased the frequencies of spontaneous chromatid breaks by 5- to 10-fold. Further, such melanoma cells expressing oncogenic BRAF were hypersensitive to UVB-induced chromatid breaks and exchanges. Published data show that oncogenic BRAF can destabilize BRCA1 interaction with chromatin, thereby attenuating BRCA1-dependent tumor suppressive functions in cell cycle checkpoints and DNA repair. Other work shows that BRCA1 protects against UV-induced genotoxicity. The data suggest that melanocytic nevi, developing in children after mutational activation of BRAF, are hypersensitive to UV-induced mutation and chromosomal damage. The results emphasize the importance of protecting children against harmful sun exposures.

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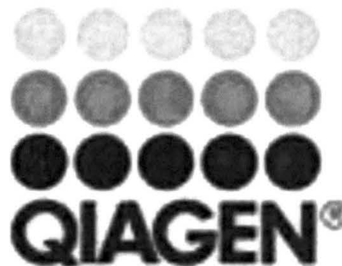

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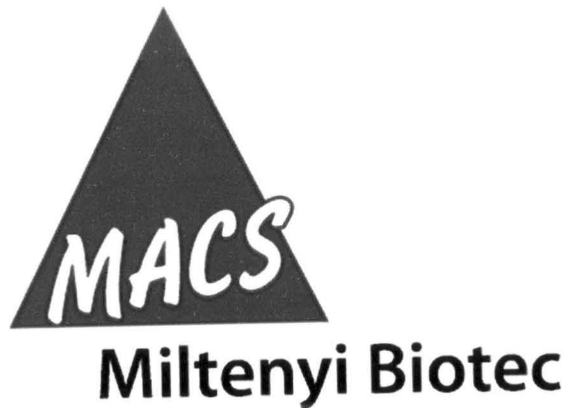

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