Carcinogenesis Specialty Section Newsletter

Society of Toxicology

In this Issue

President's Message

By: Barbara Shane

SOT-2009 in Baltimore promises to be an exciting and enjoyable meeting. I look forward to seeing all of you at the CSS reception scheduled for Tuesday evening March 17th from 6-7:30 PM where we will greet old friends and meet new ones, enjoy refreshments and congratulate the student and post-doctoral winners of the CSS awards. Winners will present a 5-minute summary of their work. At the reception we will also discuss proposed symposia, workshops etc, for the 2010 meeting in Salt Lake City.

One of the strengths of SOT is the involvement of the membership in developing the Symposia and CE courses and thus crafting the program for each annual meeting. We should take advantage of this situation and propose the most interesting and exciting symposia. For the Baltimore meeting and again for the 2010 meeting the Executive Committee of SOT has developed themes around

which the meeting will be structured. This does not mean that if your area of interest or expertise is not in these areas that your symposium will not be acceptable to the program committee. The content of the meeting depends on each of our participation in developing symposia. Please E-mail your idea(s) to Charlene McQueen (mcqueen@auburn.edu), Vernon Walker (v.walker@biomosaics.com) or Barbara Shane (shane@niehs.nih.gov) or bring your proposals to the annual meeting and we can discuss them at the mixer. Most of the officers of CSS have participated or chaired symposia and CE courses, and would be delighted to work with you in designing the proposals and suggesting appropriate co-chairs or speakers. An outline of the themes for the 2010 meeting is included in the newsletter.

This will be my last message as the president of CSS, as Charlene McQueen will be taking over after the Baltimore meeting. I have enjoyed representing all of you as the section president, and would like to thank all the members of the Carcinogenesis Specialty Section who have participated in this year's activities. We have sponsored a great slate of symposia and CE courses (8 total), for the Baltimore meeting and the strong efforts of the presenters in putting together these symposia are very much appreciated.

I wish to thank the Officers of the CSS for their contributions throughout the year to the Specialty Section. I especially want to thank Vernon for being the point of contact for this year's program. He collected and collated the symposia and workshops etc. and made sure the voting for the different topics was done correctly, to Ivan for his coordination and collection of the student and postdoctoral submissions for awards at the meeting and for Charlene for organizing a slate for our next Board for 2009-2010. Drew has done an outstanding job in recruiting interesting contributions from members for the newsletter and I for one have really enjoyed reading these summaries. I also want to thank Janet for her help with the website and communicating with headquarters about relevant changes and our student and post-doctoral representatives Supraja and Susan for representing the CSS on the student advisory council and postdoctoral assembly.

Barbara

The themes for SOT-2010 are: Cell Signaling, Gene-Environment Interactions, Metabolic Disease, Mitochondrial Basis of Disease, and Toxicity Testing in the 21st Century. Complete descriptions of each theme are provided below.

Cell Signaling - Cell signaling encompasses the broad range of pathways involved in how cells detect and respond to external stimuli and communicate with other cells. Key cellular responses regulated by cell signaling include: cell death, differentiation, and cell motility. Understanding the contribution of cell signaling pathways to toxicity is often key to determining mechanisms of toxicity or the pathogenesis of biological responses elicited by chemicals or pharmaceuticals. Furthermore, integrative systems biology approaches are being used to predict interactions between cell signaling pathways that may lead to cytotoxicity. Sessions featured in this theme will highlight mechanistic roles for cell signaling pathways in toxic responses and disease pathogenesis.

Gene-Environment Interactions - Biological research has undergone a radical evolution, a consequence of enabling technologies that permit the acquisition of data on a scale heretofore unthinkable. Access to the human genome is now readily available. Despite these impressive leaps in our knowledge base, it is clear that disease susceptibility cannot be attributed only to variations in the human genome. Additional variables exist that, in combination with our genetic background, define individual susceptibility to disease. The major variable is the environment to which our genes continually respond. Recognition of gene/environment interactions has led to increased interest in dissecting the genetic and environmental components of complex human disease. Therefore, a more precise determination of the influence of environmental exposures within a given genetic background on disease processes will be required to significantly improve the ability to predict, detect, treat and monitor disease progression and disease response. The Gene/Environment theme has been selected to highlight recent advances in this field that are relevant to the toxicological sciences.

Metabolic Disease - Metabolism is the process that provides energy for cellular functions. Metabolic dysfunction, either acquired or inherited, affects biochemical reactions resulting in metabolic diseases. The incidence of acquired metabolic diseases is rising at an alarming rate. Perturbation of lipid and glucose metabolic pathways increases the risk of developing a number of chronic conditions such as obesity, diabetes, fatty liver disease and cardiovascular disease. While genetic variability plays a role in individual susceptibility, there is evidence that environmental agents, drugs and other toxicants are contributing factors. This theme will focus on the mechanistic changes in glucose and lipid metabolism induced by toxicants and the relationship to disease progression.

Mitochondrial Basis of Disease - Mitochondrial dysfunction has been found to be an important component in the progression of numerous human disease states. In addition, the mitochondrial genome is susceptible to oxidative stress and mutation due to the high percentage of coding DNA and its small size. Therefore, the mitochondria are a suspected target organelle of xenobiotics in different model organisms. This thematic area will highlight studies that evaluate the effect of xenobiotic exposure on mitochondrial function and the connection to the progression of disease.

Toxicity Testing in the 21st Century - This theme captures research that addresses the critical need articulated in NRC's 2007 report - "Toxicity Testing in the Twenty-First Century, A Vision and a Strategy" - for development and validation of predictive high through put assays to replace current expensive and time-consuming animal tests. It is projected to include applications of genomics and in vitro tests to identify pathways of toxicity, and methods for using advanced computer power that make it feasible to analyze large volumes of complex data and use common data platforms to link existing and new exposure and effects databases.

2008-2009 CSS Officers and Student Representatives

President:	Barbara Shane (shane1@niehs.nih.gov)
Vice President:	Charlene McQueen (mcqueen@auburn.edu)
Vice President-elect:	Vernon Walker (v.walker@biomosaics.com)
Secretary/Treasurer:	Drew Burdick (andrew.burdick@pfizer.com)
Senior Councilor:	Ivan Rusyn (ivan_ruysn@unc.edu)
Councilor:	Barbara Parsons (barbara.parsons@fda.hhs.gov)
Councilor:	Mark Miller* (msmiller@wfubmc.edu)
SAC Representative:	Supraja Narasimhan
Postdoctoral Representative:	Susan Tilton
Web Liaison:	Yu (Janet) Zang

** Past President*

Important Dates

March 2, 2009	Late Breaking Abstract Deadline for SOT 2009
March 15, 2009	Student/Postdoctoral Mixer, Sunday Night 7:30-8:30PM, Convention Center Camden Lobby
March 17, 2009	CSS Officer's Breakfast, Tuesday Morning 7:00-8:30AM, Convention Center Room 339
March 17, 2009	CSS Reception, Tuesday Night 6:00-7:30PM, Convention Center Room 339
April 30, 2009	Proposal Outlines for SOT 2010 Symposia, Workshops, Roundtables, or CE Courses Due

Students and Postdocs: Join your peers and colleagues at the following events during the 2009 SOT Annual Meeting in Baltimore. Check your program for location information.

Event	Date	Time
Continuing Education Courses*	Sunday, 3/15	All day
Student/Postdoctoral Fellow Mixer*	Sunday, 3/15	7:30-8:30 PM
In Vitro Toxicology Luncheon*	Monday, 3/16	12:15-1:30 PM
Grantsmanship Forum: Tools and Skills Needed to Navigate Toxicology Research Funding	Monday, 3/16	4:35-5:55 PM
Postdoctoral Assembly Luncheon*	Tuesday, 3/17	12:00-1:15 PM
The Future of Environmental Health Science: Featuring NIEHS-Funded Early Career Investigators	Tuesday, 3/17	12:00-1:20 PM
CSS Meeting Reception	Tuesday, 3/17	6:00-7:30 PM
Toxicologists: The Next Generation	Wednesday, 3/18	7:30-8:50 AM
Career Opportunities and Transitions in Toxicology	Wednesday, 3/18	4:30-5:50 PM
Lunch with an Expert*	Sign up online: http://www.toxicology.org/ai/spd/lunchexpert.asp	

(*) You need to register in advance for these activities.

2009 CSS Student and Postdoctoral Awards

The CSS Officers would like to congratulate the student and postdoctoral award winners for 2009 that were selected from a large pool of outstanding submissions. Please show your support to our students and postdocs by visiting them at their posters. Remember to encourage graduate students and postdoctoral fellows to submit their abstracts for consideration in 2010. To qualify, your work must be related to the field of carcinogenesis. The due date for submission will be around October-November 2009.

1st Prize ($500): Michael Borland (The Pennsylvania State University). *PPARβ/δ regulates AhR signaling in skin.*

2nd Prize ($300): Jessica Graham (EOHSI / Rutgers). *The Rat Homolog of the Drosophila FRY Gene Encodes a Putative Mammary Cancer Susceptibility Locus.*

3rd Prize ($100): Kun Lu (The University of North Carolina). *Identification of Glutathione-DNA Adducts Induced by Formaldehyde.*

Postdoctoral Award ($500): Xuefeng Ren (UC Berkeley). *Alteration of H4K16 acetylation via Sas2 in yeast and MYST1 in humans is associated with increased sensitivity of arsenic toxicity.*

2009 SOT Scientific Sessions Endorsed by the Carcinogenesis Specialty Section

Continuing Education:

- AM03 Characterizing Modes-of-Action and Their Relevance in Assessing Human Health Risks

Symposia:

- MicroRNAs in Biology and Toxicology
- Genomic, Non-Genomic, and Epigenetic Mechanisms of Nuclear Hormone Receptor Action
- Nitrative and Oxidative Stress in Toxicology and Disease *
- Epigenetic Implications for Toxicology *
- Mammalian Retrotranspositional Elements: Epigenetic Regulation, Species Differences, and Potential Roles as Mediators of Cellular Responses to Toxic Stress

Workshops:

- Safety of High-Intensity Sweeteners: Bittersweet Controversy

Roundtables:

- Preclinical Evaluation of Cancer Hazard and Risk of Biopharmaceuticals

* *Primary Sponsor*

Science Spotlight: First Place Student Submission

PPARβ/δ regulates AhR signaling in skin

By: Michael Borland, The Pennsylvania State University, Center for Molecular Toxicology and Carcinogenesis. Advisor: Jeffrey M. Peters, PhD

The World Health Organization (WHO) has estimated that one in three cancers diagnosed worldwide is skin related, and, the American Cancer Society has identified skin cancer as the most common form of cancer in the United States with an estimated one million annual diagnoses (1,2). With the incidence of worldwide skin cancer growing vastly, understanding the causes and molecular mechanisms of skin carcinogenesis has become of great importance. One common mediator of skin carcinogenesis is chemical exposure, and "classic" skin chemical carcinogens are polycyclic aromatic hydrocarbons (PAHs) that are known ligands for the aryl hydrocarbon receptor (AhR).The AhR signaling pathway mediates PAH-dependent carcinogenesis by upregulating expression of the phase I cytochrome P450 (CYP) enzymes that hydroxylate PAHs (Reviewed in 3). Although carcinogen hydroxylation by CYPs is a prerequisite step to metabolism and clearance, this process generates diol-epoxide bioactivated PAHs that can form DNA adducts (Reviewed in 3). Concurrent with phase I enzyme induction is activation of phase II enzymes by AhR and the oxidant stress sensor NF-E2-related factor-2 (Nrf2); the purpose of which is to detoxify bioactivated carcinogens and reduce oxidative stress (Reviewed in 3,4). This two-step carcinogen metabolic pathway requires a crucial balance between phase I and II enzymes to detoxify carcinogens before the PAH-dependent genotoxic effects can occur. This coordinated regulation of phase I and II enzymes by two distinct, yet inter-regulated, systems (AhR and NRF2) also demonstrates the importance of responding to environmental toxicants/carcinogens and the importance of balancing carcinogen bioactivation and clearance.

Peroxisome proliferator-activated receptors (PPARs) are ligand activated transcription factors involved in a multitude of biological processes. Three PPAR isoforms (α, γ, and β/δ) have been identified, and each mediates tissue-specific biological responses. While much is known about the functions of PPARα and PPARγ, much less is known about the biological function of PPARβ/δ. The PPARβ/δ-null mouse model system has demonstrated that PPARβ/δ protects against phorbol

ester skin hyperplasia and attenuates skin tumorigenesis in a two-stage skin carcinogenesis bioassay (Reviewed in 5). While the hyperproliferative phenotype of PPARβ/δ-null keratinocytes could account for previously observed differences in skin tumorigenesis (6), PPARβ/δ could also alter carcinogen metabolism and genotoxic initiation. This hypothesis was tested in mouse skin, and surprisingly, PPARβ/δ-null mice were refractory for PAH-dependent induction of phase I enzymes. It was then hypothesized that PPARβ/δ alters carcinogen metabolism by modulating the AhR-dependent signaling pathway. Direct evidence from primary mouse keratinocytes has shown that the absence of PPARβ/δ significantly attenuates PAH-dependent induction of phase I enzymes, and this observation identifies the basal layer of the skin as the effector cell type. This modulation was examined in response to PAHs of varying structure, and the attenuation of AhR phase I signaling was still evident in PPARβ/δ-null keratinocytes. Surprisingly, while AhR-dependent regulation of phase II enzyme mRNAs was also found to be modulated in PPARβ/δ-dependent manner, AhR target genes distinct from phase I and II metabolism were not found to be regulated by PPARβ/δ. NRF2 is also known to mediate phase II enzyme mRNA induction (Reviewed in 7); consequently, the expression of NRF2 and a non-AhR regulated phase II enzyme were examined, and both genes responded to PAH in a PPARβ/δ-dependent manner. Furthermore, the basal expression of several phase I and II enzymes appears to be modulated by PPARβ/δ, and this observation is being more closely examined. AhR signaling was also examined in liver, and surprisingly, phase I enzyme induction was not PPARβ/δ-dependent. This observation, in conjunction with the described keratinocyte observations, demonstrates that PPARβ/δ-dependent modulation of AhR signaling is skin-specific and that the primary mouse keratinocyte model will be extremely useful in delineating the mechanism by which AhR signaling is modulated by PPARβ/δ.

With a well-described mRNA phenotype of PPARβ/δ-dependent regulation of AhR signaling in the skin, the functional and biochemical properties of the AhR signaling pathway were more closely examined. The constitutive expression of AhR and accessory proteins were first examined and no differences in protein expression were observed. The ability of the AhR to bind ligand and translocate to the nucleus was examined using irreversible or reversible radioligands, and again, there were no observed differences between wild-type and PPARβ/δ-null primary keratinocytes. The presence of a physical interaction between the AhR and PPARβ/δ was also investigated using an overexpression GST-

pulldown system, and preliminary evidence suggests there is not a direct interaction between these receptors. The promoter occupancy of CYP1A1 was subsequently examined using chromatin immunoprecipitation (ChIP), and interestingly, the absence of PPARβ/δ significantly reduced AhR binding and histone acetylation in response to PAH. This suggests PPARβ/δ may modulate coactivator recruitment or alter higher order chromatin structure at AhR target gene promoters. Further studies are underway to identify the coactivator(s) that are differentially recruited to promoters in response to PAH and to determine if chromatin structure or post-translational modifications mediate this effect. The end product of carcinogen bioactivation is DNA adduct formation, and preliminary data also suggests there is decreased adduct formation in PPARβ/δ-null keratinocytes. To correlate human risk to the observed murine modulation of AhR signaling, a human model system was devised using the nontumorigenic human keratinocyte cell line HaCaT (8). HaCaT stable cell lines were created that express either an shRNA targeted against PPARβ/δ, or a non-targeted control. Preliminary evidence has demonstrated that the absence of PPARβ/δ also attenuates PAH-dependent induction of phase I enzymes. These results demonstrate that PPARβ/δ modulates the human and mouse AhR signaling pathway and that coactivator recruitment or chromatin modifications mediate this effect. Future studies will focus on identifying and verifying the molecular mechanism by which PPARβ/δ modulates AhR signaling in human and mouse keratinocytes.

This project has focused on describing and characterizing PPARβ/δ-dependent modulation of the AhR signaling pathway in the skin, and future studies will focus on identifying the mechanism of modulation and characterizing the carcinogenic risk of this modulation. This characterization will include temporal analyses of the AhR signaling pathway and functional analysis of examine if the activation status of PPARβ/δ (unliganded, liganded, antagonized) alters PAH-dependent responses. The larger perspective goal of these studies is to understand the consequences of altered AhR signaling in skin carcinogenesis. If AhR signaling is not mediating carcinogen metabolism (in PPARβ/δ-null mice), it will be important to determine what, if any, mechanisms are supplanting AhR signaling and the final metabolic consequences of this alternative mechanism. These questions will be addressed in wild-type and PPARβ/δ-null primary keratinocytes using microarray technology and metabolomic profiling. Another consequence of PAH metabolism is DNA adduct formation, and genotype-specific differences in DNA adduct formation need to be more thoroughly analyzed to validate the previously described differences in adduct formation. The biological

significance of markedly different carcinogen metabolism and DNA adduct formation is currently being examined in a complete carcinogen bioassay using in wild-type and PPARβ/δ-null mice to determine whether altered AhR signaling significantly alter tumor onset, incidence, size, or malignant conversion. While substantial progress has been made characterizing the molecular modulation of AhR signaling by PPARβ/δ, this research still requires further in vivo validation, a clear delineation of murine carcinogenic risk, and a correlation to human risk. Importantly, since no functional polymorphisms in the AhR have ever been identified to account for the wide variation in P450 inducibility in humans, it is possible that this research may uncover a new variable that could contribute to human skin carcinogenesis. Overall, this project describes a novel, epigenetic interaction between two ligand-activated transcription factors, and it is believed that this interaction influences the balance between carcinogen metabolism and bioactivation in the skin. The fact that PPARβ/δ may also play a role in antioxidant responses also signifies the importance in understanding the biological functions of PPARβ/δ. As knowledge of the biological function of PPARβ/δ has been lacking, this research enhances our understanding of PPARβ/δ biological function and describes an interaction with another receptor systems that could challenge our understanding of chemical skin carcinogenesis.

References:

1. World Health Organization (WHO). (2001) Fact sheet N°261. WHO Media Centre.
2. American Cancer Society. (2008) Cancer Facts & Figures 2008. Atlanta: American Cancer Society.
3. Ramadoss et al. (2005) Expert opinion on drug metabolism & toxicology 1(1), 9-21.
4. Kwak et al. (2004) Mutation Research 555(1-2), 133-148.
5. Burdick et al. (2006) Cellular Signaling 18(1), 9-20.
6. Borland et al. (2008) Molecular Pharmacology 74(5), 1429-42.
7. Xu at al. (2005) Archives of Pharmacal Research 28(3), 249-68.
8. Boukamp et al. (1988) Journal of Cell Biology 106(3), 761-71.

Science Spotlight: First Place Postdoctoral Submission

Histone Acetylation and Susceptibility to Arsenic Toxicity

By: Xuefeng Ren, William J. Jo, Maria Aleshin, Henri Wintz, Martyn T. Smith, Chris D. Vulpe and Luoping Zhang (Division of Environmental Health Sciences, School of Public Health, University of California, Berkeley, CA).

Consumption of drinking water contaminated with high levels of inorganic arsenic (AsIII) is a worldwide public health concern as chronic exposure to AsIII causes multiple deleterious health effects, including various cancers. The mechanisms by which arsenic leads to tumorigenesis are not fully understood. Chronic exposure to AsIII leads to extensive changes in global gene expression without genomic mutation (Andrew et al., Env. Health Persp. 2008 (116) 524), suggesting that epigenetic changes play a critical role in arsenic-induced carcinogenesis.

In a genome-wide phenotypic screen of yeast genes, we identified the heterotrimeric something about silencing 2 (Sas2) as being required for optimal growth in AsIII. Sas2 and its human ortholog, MYST1, encode histone acetyltransferases responsible for the acetylation of H4K16 in yeast and human cells, respectively. Histone acetylation is essential for the establishment of transcriptionally competent chromatin. Hypoacetylation of H4K16 is commonly found in human tumors and cell lines, and loss of acetylation at histone 4 (H4) appears to occur during malignant transformation (Fraga et al., Nat. Genetics 2005 (37) 391). Since altered histone acetylation, especially histone H3, and expression of associated genes were recently shown to occur during arsenical-induced malignant transformation, we hypothesized that H4K16 acetylation may also be involved in AsIII-induced tumorigenesis.

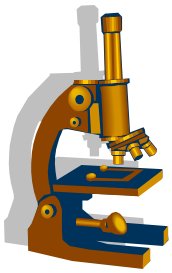
In order to explore the potential role of H4K16 acetylation in the toxicity of AsIII and its metabolite, monomethylarsonous acid (MMAIII), we knocked down MYST1 in human uroepithelial UROtsa cells using lentivirus-based RNA interference (RNAi) and subsequently exposed the cells to AsIII and MMAIII. In our SOT abstract we report that partial silencing of MYST1 significantly increases the sensitivity of UROtsa cells to both AsIII and MMAIII and results in a considerable reduction in H4K16 acetylation. This suggests that the bulk of H4K16 acetylation is catalyzed by the MYST1 protein in UROtsa cells as shown in other cell lines (Taiplae et al., Mol. Cell. Biol.

2005 (25) 6798), and that decreased H4K16 acetylation could cause increased sensitivity to arsenic. Further, it indicates a common mechanism(s) by which both AsIII and MMAIII exert toxicity, despite the fact that MMAIII is more potent. To further investigate the possible role of H4K16 acetylation in AsIII resistance, we evaluated the growth phenotype of a H4K16→R yeast mutant, in which a non-acetylatable arginine residue replaces lysine at position 16. This strain exhibited slow growth compared to the wild type and was sensitive to high doses of AsIII. These results demonstrate that reduced H4K16 acetylation led to the observed increase in sensitivity to AsIII in the yeast mutant, and suggest that altered H4K16 acetylation is associated with the AsIII toxicity.

In conclusion, we have shown that MYST1 plays a role in human cellular susceptibility to arsenic toxicity through its H4K16 acetylation function. MYST1 was also shown to modulate the effects of ionizing radiation on DNA damage through effects on H4K16 acetylation and double-strand break repair (Gupta et al., Mol. Cell Biol. 2008 (28) 397; Taiplae et al., Mol. Cell. Biol. 2005 (25) 6798). Through the acetylation of H4K16, MYST1 likely promotes or maintains adequate expression of genes involved in the response to arsenic-induced stress. Our data confirm a role for altered epigenetic regulation in arsenic-induced toxicity and tumorigenesis. Thus, a comprehensive examination of epigenetic aberrations and identification of epigenetically labile genes associated with arsenic exposure represents a critical avenue for the identification of biomarkers of exposure and susceptibility to arsenic.

Action Required!

It is time to begin planning for the 2010 annual SOT meeting in Salt Lake, UT. Program proposals for the 2010 meeting will be due soon (April 30). If you have suggestions for symposia, workshops, roundtables or CE courses, please contact Charlene McQueen (mcqueen@auburn.edu), Vernon Walker (v.walker@biomosaics.com) or Barbara Shane (shane@niehs.nih.gov). Alternatively, bring your suggestions to the Carcinogenesis mixer, where proposed topics for next year's sessions can be discussed during the business meeting.



Would you like to highlight a recent advance or scientific opinion in an upcoming CSS Newsletter? Please submit articles to Drew Burdick (Andrew.Burdick@pfizer.com) by April 15, 2009 for inclusion in the Spring Newsletter. A special thank you to Michael Borland and Xuefeng Ren for supplying articles for this edition!

Finally, please encourage your graduate students, postdoctoral fellows, and SOT colleagues to join and renew their Carcinogenesis Specialty Section membership. Remember that students and postdocs receive the first Specialty Section membership at no cost. Your continued contribution and support ensures that this remains an outstanding specialty section!