

# ***Carcinogenesis Specialty Section Newsletter***

## **Society of Toxicology**

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### **President's Message**

*By: Barbara Shane*

I would to thank those members and officers of the CSS who were involved in the CSS during the past year, especially those officers who encouraged members to submit proposals for symposia and workshops for the meeting in Seattle. I would especially like to thank Charlene McQueen for coordinating the receipt of proposals. CSS sponsored 3 symposia, 1 workshop, and 1 roundtable discussion as the primary SS and two symposia and three workshops as the secondary

endorser. Congratulations to those whose symposium was selected by the program committee.

We appreciated the submission of symposia and a CE course from the membership for the Baltimore meeting in March 2009 and thank Vernon Walker for coordinating the submission of the symposia etc. for the next meeting. I would like to encourage members to resubmit their symposia for the 2010 meeting if the Program Committee does not accept

your submission for the 2009 meeting. Bear in mind that SOT is especially interested in focused areas for symposia and each year plans to encourage submission in these particular areas. For the 2009 meeting, the program committee was especially interested in symposia in biomarkers, epigenetics, inflammation and disease, neurodegenerative diseases and nanotechnology, but of course any good submission in other areas would be considered. When developing symposia and CE courses, consider proposing your post-docs as co-chairs for sessions. For the Baltimore meeting we have submitted 8 symposia as the primary SS and 6 as a secondary sponsor. There are only about six weeks from the date of the SOT meeting in March and the end of April when the submission of symposia to the program for the next meeting is required. We already have a suggested symposium for the 2010 meeting so it is never too early to be thinking of what you would like include in the program. Please contact Charlene, Vernon or myself with any suggested topics or even a partial fleshed out symposium or CE course for our review and help if you need it. The more information you have regarding the abstract for the symposium, including the participants their titles and individual abstracts, the more likely the program committee will look favorably on your proposal.

I would also like to thank those officers who rotated off the CSS for

their contribution to the running of the CSS namely, Michelle Hooth for her stellar organization in coordinating the abstracts from students and post-docs for the CSS awards; Chris Corton for compiling the newsletters and keeping up with our expenditures for the past three years, and to Mark Miller who kept us in order reminding us when certain deadlines were approaching. I know this is early summer but think about the abstracts for your students and post-docs for the Baltimore meeting, as the submission date is November 16, 2008. Also, please encourage your students and post-docs to join the CSS; they can be members of more than one specialty section and there are no dues for the first SS they join.

I would like to congratulate our new CSS officers for the 2008-2009 year namely Barbara Parsons (councilor), Vernon Walker (vice-president elect) and Andrew Burdick (secretary and treasurer), Supraja Narasimhan (student representative) who won the first prize for the best abstract and Susan Tilton (post-doc representative). Janet Zu who was our last year's post-doc representative has agreed to stay on the executive committee as our web liaison. If you find any errors on our website or you have any suggested items you think should be on the website, please send the information to Janet who will pass it on to the executive committee for their approval to have it posted. Many thanks to those members who ran for office last year, namely,

Kathleen Dougherty, Martin Ronis and John Wise. We hope you as well as others will be willing to run for the upcoming slate. If you are interested in running you may nominate yourself. Send your name and email address to Mark Miller who will be coordinating the slate for next year.

We had a good turnout at the SOT CSS reception on Tuesday evening and hope you had an enjoyable time renewing acquaintances and meeting new colleagues. If you have any suggestions as to how you would like the reception to be structured for the 2009 meeting, do not hesitate to contact me. We want the membership to enjoy the reception. For the last couple of years, we have asked the student and postdoctoral winners to give five-minute presentations of their work, as we think this is a good opportunity for the students to discuss their work in a friendly atmosphere and for the

membership to hear about their research.

The CSS sincerely thanks Dr. Ganesh Balasubramanian from Merck for the company's gracious donation to the CSS of the Merck Award. We are extremely grateful for this award, as is the graduate student who won the award, Leigh Greathouse. Congratulations!

For this newsletter Drew was able to obtain interesting articles from two of our members. Please send him or me any summaries of meetings you attend or other items that you think will be of interest to the CSS for inclusion in the next newsletter. I look forward to working with you throughout the year. Please do not hesitate to contact me or any members of the executive committee with any suggestions or items of interest you would like to be included in the next newsletter

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## 2008-2009 CSS Officers and Student Representatives

<b>President:</b>	Barbara Shane (NIEHS)
<b>Vice President:</b>	Charlene McQueen (Auburn)
<b>Vice President-elect:</b>	Vernon Walker (LRRRI)
<b>Secretary/Treasurer:</b>	Drew Burdick (Pfizer)
<b>Senior Councilor:</b>	Ivan Rusyn (UNC)
<b>Councilor:</b>	Barbara Parsons (USFDA)
	Mark Miller* (Wake Forest)
<b>SAC Representative:</b>	Supraja Narasimhan (Boston Univ)
<b>Postdoctoral Representative:</b>	Susan Tilton (Fred Hutchinson)
<b>Web Liaison:</b>	Yu (Janet) Zang (Johns Hopkins)

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## Important Dates

October 3, 2008	SOT Abstract Submission Deadline
November 16, 2008	Deadline for 2009 CSS Specialty Section Student and Postdoctoral Award Submissions

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## 2008 CSS Student and Postdoctoral Awards

The CSS Officers encourage graduate students and postdoctoral fellows to submit their 2009 abstracts for competition for best abstract awards. To qualify, your work must be related to the field of carcinogenesis. The due date for submission is extended to November 16, 2008. Abstracts and a recommendation letter from your advisor (not to exceed 2 pages) can be sent electronically to Dr. Ivan Rusyn ([iir@unc.edu](mailto:iir@unc.edu)). Check the SOT website for additional information on student awards. Join us in congratulating the 2008 Award Winners:

- 1st Prize (\$500):** Supraja Narasimhan (Boston University School of Medicine, Boston MA). *Role of the Aryl Hydrocarbon Receptor in Mammary Tumor Progression.*
- 2nd Prize (\$300):** James A. Jacobus (University of Iowa, Iowa City, IA). *The End Draws Near: Telomere Shortening Induced by a Quinone Metabolite of PCB3.*
- 3rd Prize (\$100):** Jennifer D. Cohen (University of Arizona, Tucson, AZ). *Constitutively Active B-Raf Does Not Regulate Cyclin D1 in 2,3,5-Tris-(glutathion-S-yl) Hydroquinone Transformed, Tuberous Sclerosis-2 Null Cells.*

**Postdoctoral Award (\$500)** : Susan C. Tilton (University of Washington, Seattle, WA). *Mechanisms of chemically-induced DNA damage in the etiology of infant leukemia.*

**Merck Award (\$1000)** : Leigh Greathouse (MD Anderson Cancer Center, Smithville, TX). *Identification of genes developmentally reprogrammed in the uterus by neonatal exposure to xenoestrogens.*



Student and postdoctoral award winners: From left to right: Supraja Narasimhan (1<sup>st</sup>), Leigh Greathouse (Merck Award), Jennifer Cohen (3<sup>rd</sup>), Susan Tilton (Postdoctoral Award), James Jacobus (2<sup>nd</sup>).

**2008 SOT Scientific Sessions Endorsed by the  
Carcinogenesis Specialty Section**

**Continuing Education:**

- Epidemiology for Toxicologists: Introduction

**Symposia:**

- New Developments in Liver Tumor Biology\*
- Environmental Influence on Female Puberty and Breast Tumorigenesis
- Molecular and Genomic Insights into the Nrf2-Regulated Oxidative Stress Response: Impact on Carcinogenesis
- New Concepts in the Etiology of Breast Cancer: From Genes to Environment and Back Again\*
- Perinatal Exposure to Nucleoside Reverse Transcriptase Inhibitors (NRTIs) Induces Transplacental Genotoxicity and Mitochondrial Toxicity\*

**Workshops:**

- To Dye or Not to Dye: Safety of Oxidative Hair Dyes
- Host Susceptibility and Chemical Safety Testing: New Approaches to Estimate Risks in the Human Population\*
- Molecular Mechanisms and Molecular Biology of Metal Carcinogenesis
- Interdisciplinary Approaches for Improving Chemical Hazard Paradigms

\* *Primary Sponsor*

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## Invited Opinion

### **Toxicology and Emergence of New Technologies in Epigenetics**

*By: Tim Beischlag, Ph.D.,  
Associate Professor, Simon  
Fraser University,  
Vancouver, B.C.*

The study of how the extra-genomic characteristics in chromatin structure affect gene expression and determine cell differentiation pathways has been the focus of developmental biologists for almost five decades. Early studies focused on how changes in chromatin structure in a given tissue coincided with certain developmental checkpoints and cell lineage fate; what was termed “cell automation”. Initially, studies were limited to simple organisms and small invertebrates such as yeast and *Drosophila*. These studies laid the groundwork for investigations with greater human health applications such as those concerning X-inactivation and aging. We now look at the epigenome as a second level of gene expression control, a dynamic landscape overlaid on a somewhat static genome. The convergence of existing and emerging technologies has led to more intense scrutiny of the epigenetic mechanisms that control gene expression.

Viewed by many, only in terms of methylated vs. un-methylated DNA, or hetero- vs. euchromatin, the mechanisms that govern the

topography of the epigenetic landscape are multi-faceted. Early studies focused (and continue to focus) on DNA methylation patterns as a predictor of chromatin status. However, investigators have broadened these studies to include the patterns of post-translational modification of histones, or the so-called “histone code”, and the role played by non-coding RNA (ncRNA) not only in directing the methylation of DNA but also in the role they play in modifying histones.

The specter of a dynamic epigenome and its role in the pathogenesis of disease is particularly exciting for the field toxicology. For decades, we have struggled with our inability to resolve an abundance of mechanistic data regarding the effectors of toxicity with the elusive molecular determinants of the associated pathologies. The field of chemical carcinogenesis offers some excellent examples. In the case of compounds that cause direct DNA damage, the mechanisms of carcinogenesis are fairly well understood. However, for compounds that do not directly lead to DNA damage, such as TCPOBOP, dioxins, PCB's and endocrine disruptors, we find ourselves in many instances still looking into the “Black Box”. For many of us, the lack of evidence pointing to genome based causalities has led to the conclusion that in fact, this other order of

organization within the genome holds the clues to the pathologies that we investigate. In the case of the chemicals listed above, all the players are known, cloned and characterized, yet the mechanisms resulting in disease remain elusive.

Therefore, increasingly we turn to epigenetics; and for good reason. Insults or pressures that alter the function or expression of histone- or DNA-modifying enzymes can cause unwanted changes in the epigenome. These changes convert chromatin from a condensed and essentially silenced state (heterochromatin) to an “unwound” state (euchromatin) that is permissive to activated gene transcription, or vice versa. Untoward alterations in chromatin structure can significantly enhance susceptibility within individuals or populations to a multitude of pathological conditions including but certainly not limited to, cancer, diabetes, susceptibility to infection, and mental disorders. Indeed, it is not just heritable changes in chromatin structure that govern generational defects that we solely are concerned with. In the simplest situation, these alterations can dictate an individual's susceptibility to future exposures of toxicological insults confronted years, even decades later. Thus, the observation that alterations in the epigenome are transferred in a heritable fashion offers potential explanations for multiple toxicological insults and the potential for new avenues of drug discovery.

The presence of methylcytosine residues within DNA is associated with compacted chromatin (or heterochromatin) and de-acetylated histones. This chromatin state is responsible for the silencing of gene transcription and is necessary for the orderly regulation of gene programs during development and for the control of gene dosage as seen in X chromosome inactivation. It has been argued that DNA methylation promotes histone deacetylation leading to compaction and vice versa. Nevertheless, it seems clear that DNA unwinding and compaction are dictated by a multi-step process of post-translational modifications of key residues within the amino-terminal tails of histones, known as the histone or combinatorial code. Furthermore, these processes and that of DNA methylation are, to a great degree, interdependent processes.

The majority of covalently modified residues found within the genome occur at cytosine-poly-guanine (CpG) islands. Bisulfite-induced modification of unmethylated cytosine residues combined with direct sequencing of single stranded DNA allows for the mapping and monitoring of DNA methylation patterns. Illumina (Solexa) sequencing provides investigators with the ability to obtain up to one gigabase of sequence information from a single run. This technology has been adapted for multiple platforms including serial analysis of gene expression (SAGE), chromatin

immunoprecipitation (ChIP), and micro-RNA applications.

More recently, RNA interference, or ncRNA-mediated post-transcriptional gene silencing has emerged as another hitherto, unrecognized gene expression control mechanism. Non-coding RNA's have been implicated in directly regulating gene expression at the level of translation (the classical RISC/Dicer mechanism) as well as modifying DNA methylation status and histone tail post-translational modifications (PTM's).

High throughput automation that combines two-dimensional fluorescence difference gel electrophoresis (2D-DIGE), nanospray mass spectroscopy/TOF analysis allows for the monitoring of PTM's of specific proteins. Increasingly, highly selective antibodies that recognize specific PTM's at distinct residues within proteins are being made available to the general research community. These tools, ever increasingly, allow biologists to dissect signaling pathways protein by protein, and now, PTM by PTM.

While different technologies and fields are converging, several key issues that will enable the field of epigenetics to move forward still exist: (i) Understanding the full

scope of ncRNA function is a field that is still in its infancy; (ii) the ability to monitor ncRNA function has not been fully developed; (iii) not all antibodies are useful in every application; and, (iv) the molecular determinants of histone function have not yet been elucidated. In addition, while the ability to rapidly sequence entire genomes exists, this technology is still prohibitively expensive for population-based studies. Nevertheless, the combination of with DNA array technologies, proteomics and the ability to monitor the production and action of non-coding RNA's provides toxicologists with the appropriate tools to broaden the scope of risk assessment and drug discovery.

As is the case for all scientists, the challenge of the modern toxicologist will be to integrate the technology that is driving emerging fields of research and incorporate those technologies into their own research programs. Over the past several decades, pharmacogenetics opened the door to pharmacogenomics, which in turn, led to the first realistic view of "personalized" medicine. The construction of an epigenetic map and a broader understanding of the dynamic nature of the epigenome will certainly help health researchers realize this goal.

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## SOT Meeting Highlight

### **Kinase inhibition: 518 opportunities or 518 ways to cause toxicity?**

*By: Andrew Olaharski, Ph.D., and Kyle Kolaja, Ph.D., DABT, (Discovery and Investigative Toxicology, Roche, Palo Alto, CA)*

Between 20-50% of the pipeline for the pharmaceutical industry is dedicated to the development of small molecule kinase inhibitors (SMKIs). Because of their fundamental role in cell cycle and signal transduction, kinases are often implicated in disease and are, as such, attractive therapeutic targets. Most drugs that target kinases often do so by their interaction with the ATP binding pocket, inhibiting the ability of the kinase to phosphorylate the intended target. Given the high sequence homology of the conserved ATP binding pocket across the 518 kinases that comprise the human kinome, SMKIs often inhibit many more kinases than what is targeted. This off-target kinase inhibition is not without its price, as SMKIs are sometimes associated with serious adverse toxicological events.

Our objective was to identify kinases that correlate with toxicity when inhibited, with the ultimate goal being to provide medicinal chemists with the information required to rationally design SMKIs devoid of

activity against kinases of concern. Two toxicities, in vitro genotoxicity and in vivo bone marrow toxicity, were investigated to develop mathematical models capable of predicting these toxicities based upon kinase inhibitory profiles.

For in vitro genetic toxicology, a mathematical model was developed to identify kinases that are critical for a positive result in the micronucleus assay. Specifically, 54 structurally diverse SMKIs screened against 290 kinases were tested in the L5178Y micronucleus assay. The mathematical model's internal prediction accuracy was estimated to be 80% and the optimal set of features was comprised of 13 kinases, including CDK2, GSK3 $\alpha$  &  $\beta$ , and PCTK1 & 2. An additional 33 SMKIs not contained in the original 54-compound training set were used for a forward validation of the model. Based upon kinase inhibition profiles alone, the model properly predicted 76% of the in vitro micronucleus results (1). For in vivo bone marrow toxicity, 65 structurally diverse SMKIs were analyzed for trends across kinase inhibitory profiles with regards to hematopoietic toxicities. Inhibition of a number of kinases was identified to be correlated with in vivo bone marrow ablation, including ANKK1, AuroraC, and Tyk2. In addition, alternative high-throughput in vitro CFC assays are being

assessed to evaluate their ability to predict in vivo toxicities and identify additional kinases that correlate with toxicity (2).

Our understanding of the toxicological ramifications that associate with inhibiting the kinome are still in its infancy. These studies are the first of several steps in realizing the ultimate goal of generating a list of kinases that are known to cause toxicity when inhibited, assisting medicinal chemists in their quest of intelligent drug design, and reducing the toxicities associated with marketed SMKIs. The early returns of this project suggest that kinase inhibition profiles can be modeled to accurately predict toxicity.

#### References:

1. Olaharski AJ, Gonzaludo N, Goldstein D, Bitter H, Kirchner S, Fischer G, Platz S, and Kolaja K (poster). Using kinase selectivity profiles to predict in vitro micronucleus assay results. The Toxicologist, p 390, March 2008. <http://www.toxicology.org/ai/pub/Tox/2008Tox.pdf>
2. Olaharski AJ, Bitter, H, Lin, H, Goldstein D, Platz S, and Kolaja K (poster). Using kinase selectivity profiles to predict in vitro and in vivo bone marrow toxicity. The Toxicologist, p 465, March 2008. <http://www.toxicology.org/ai/pub/Tox/2008Tox.pdf>

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### Action Required!

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 December 15, 2008 for inclusion in the Winter Newsletter. A special thank you to Tim Beischlag and Andrew Olaharski for supplying articles for this issue!

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!

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