



## Fall 2012 Newsletter

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## President's Message

*By: Helmut Zarbl*



There has never been a more exciting time to be a cancer researcher than the present. This coming year, 2013, will be the 60<sup>th</sup> anniversary of Watson and Crick's amazing insights that finally helped unravel the structure of DNA. It took another 50 years to complete the first draft sequence of human genome. Over the last two decades we also have added enormous insight into the role DNA methylation, chromatin modification and non-coding RNAs play in epigenetic regulation of gene expression. As a result of the technologies spawned by the genome project, including massively parallel, Next Generation DNA sequencing technologies and commensurate leaps in bioinformatic and computing capabilities, it is now possible to re-sequence an entire human genome and define associated genome wide epigenetic modifications in a matter of days. Analytical chemistry has likewise added orders of magnitude in sensitivity and throughput. Computational chemistry and QSAR methodologies have also advanced at incredible rates, making structure-function predictions more robust. The dual pronged approach of exposure science and exposomics herald the integration of exposure and biological responses as never before.

What does access to all this new knowledge and these amazing tools mean for toxicologists who are interested in carcinogenesis? Chemical carcinogenesis is focused on genotoxic compounds and genes that alter sensitivity to genotoxicants. High-throughput DNA sequencing tools now allow for assessment of sequence variation on a genomic scale, enhancing our ability to identify susceptibility genes and vulnerable individuals and populations.

As we unravel the key role of epigenetics in cancer susceptibility, promotion and mutagenesis, it is clear that we must also consider a new class of carcinogens, **epigenotoxicants**. These are chemicals and stressors that change chromatin marks directly, or indirectly, by effects on the activities of enzymes ('writers' and 'erasers'), 'readers' and 'remodelers' that maintain, induce, modulate and transduce epigenetic changes. Epigenotoxicants provide exciting new research opportunities, but also present new challenges for carcinogenicity testing, risk assessment, risk management and

regulatory policy.

The ability to more accurately assess and model exposures in the context of the exposome, defined as relating exposures and biomarkers of total exposure occurring across a life span of the host to outcomes. The microbiome, diet, drugs, lifestyle, other environmental stressors, and co-morbid disease are also poised to revolutionize carcinogenesis research, testing and risk assessment.

As we embrace a new area of research in carcinogenesis, we need to focus our efforts not only on the emerging science and technologies, but also on the need to train a new cadre of interdisciplinary toxicologists. Part of our mission must be to ensure that our trainees have access to transdisciplinary programs that prepare them for careers that in addition technological savvy, will also require intimate knowledge of chemistry, quantitative biology, toxicology, and bioinformatics. The magnitude of this challenge cannot be underestimated. Those of us engaged in carcinogenesis research and teaching must take up the torch and make sure we remain at the cutting edge. Our ability to educate and prepare our students for successful careers in carcinogenesis begins with educating ourselves. Fortunately SOT has always been a leader in education for both our young trainees and seasoned veterans eager to learn and adopt emerging science. Attending the SOT Annual Meeting is a wonderful social experience, an opportunity to meet new colleagues and old friends and a venue for our students and trainees to present our most recent research. But it is more than that. The annual meeting offers Scientific Sessions, Symposia, Workshops and Continuing Education Courses on emerging science and technology applications. Lifelong learning is one of the most rewarding, challenging and enjoyable privileges of being a researcher. Join the fun and get involved in CSS and SOT activities. Submit an abstract, organize a session, attend or organize a Continuing Education course, organize a meeting on Contemporary Topics in Toxicology, participate in Chat with an Expert, or nominate a good student or colleague for an award. You only get out of our Specialty Session what you put into it. See you all in San Antonio!!!!

Helmut Zarbl, Ph.D., ATS

## 2011-2012 CSS Officers and Representatives:

- President:** ..... Helmut Zarbl ([zarbl@ehsi.rutgers.edu](mailto:zarbl@ehsi.rutgers.edu))
- Vice President:** ..... Miriam C. Poirier ([poiriern@dc37a.nci.nih.gov](mailto:poiriern@dc37a.nci.nih.gov))
- Vice President-elect:** ..... Elaine Faustman ([faustman@u.washington.edu](mailto:faustman@u.washington.edu))
- Past President:** ..... David E. Williams ([david.williams@oregonstate.edu](mailto:david.williams@oregonstate.edu))
- Secretary/Treasurer:** ..... John Wise ([john.wise@usm.maine.edu](mailto:john.wise@usm.maine.edu))
- Councilor:** ..... Yvonne Dragan ([yvonne.dragan@astrazeneca.com](mailto:yvonne.dragan@astrazeneca.com))
- Councilor:** ..... Stephen Safe ([ssafe@cvm.tamu.edu](mailto:ssafe@cvm.tamu.edu))
- Student Representative:** ..... Sara Nowinski ([sara.nowinski@gmail.com](mailto:sara.nowinski@gmail.com))
- Postdoctoral Representative:** Gayathri Chadalapaka ([gchadalapaka@cvm.tamu.edu](mailto:gchadalapaka@cvm.tamu.edu))



Yvonne Dragan



Elaine Faustman



Sara Nowinski



Miriam Poirier



Stephen Safe



David Williams



John Wise



Helmut Zarbl

## Upcoming Deadlines:

Nominations for 2013 CSS Student and Postdoctoral Awards:	November 16, 2012
2013 Award Nominations:	October 9, 2012
Early Bird Registration:	January 25, 2013
Housing Reservation:	February 15, 2013
Cancellations:	February 15, 2013
Session Proposal for The 2014 Annual Meeting:	April 30, 2013

## **A New Logo and an Endowment for CSS**

*By: Helmut Zarbl*

You may have noticed a new logo at the top of the Newsletter. Many Specialty Sections and Special Interest Groups have logos for use on official documents and communications. Logos are important for any organization, providing brand recognition, professionalism and a sense of community. As the president, I have taken it upon myself to work with a team of graphic artists to design a logo for the CSS, at my expense. The logo incorporates a crab, the universal symbol for the dreaded disease we study, holding a molecule of benzo[a]pyrene, a carcinogenic component of soot, the first chemical carcinogen recognized by Perceval Potts in 1775. I hope you agree that it is a fitting logo for or specialty section.

If you attend the Annual meeting you may have noticed that many other Specialty Sections and SIGs have incorporated their logos into pins, which they wear proudly on their lapels or name badges. The logos identify other members, advertise the groups, and lead to conversations, new collaborations, collegiality and friendships. To thank all of you for giving me the honor of serving as your president, I am having lapel pins made at my expense and will distribute them to you free of charge at the CSS reception in San Antonio!!!

But there is a catch. In return, all that I ask is that every member of CSS consider making a contribution towards a new named CSS President's Endowment Fund. If we raise \$50,000, SOT will manage the fund and all proceeds from the endowment will then support the important work of the CSS (student and postdoc awards, travel awards, etc) in perpetuity. We have approximately 270 members in CSS. If each of us makes a onetime, tax deductible donation of \$200, we will exceed this goal. Please consider being part of a revitalized and proactive Specialty Session, which will help attract new researchers to our society and the important field of chemical carcinogenesis. So, the catch is to consider donating to endowment, but even if you choose not to contribute to the new endowment, the pin is still my gift to you!!!!

**Time to Apply for the 2013 CSS Student or Postdoctoral Award: Due November 16, 2012!**

The CSS Officers encourage graduate students and postdoctoral fellows to submit their 2013 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 November 16, 2012. Applicants should submit an electronic version of their abstract, a 1–2 page narrative describing their research hypothesis, background and significance, and a letter of recommendation from their advisor (not to exceed 2 pages) as a .pdf document to [Dr. Yvonne Dragan](#).

The First Place student winner will receive the Dharm V. Singh Endowment Award. He/she will receive a plaque and check (amount TBD). The Dharm V. Singh Endowment Award winner will be asked to be the Student Representative for the CSS. Second, third and fourth place graduate student winners will receive plaques as well as checks for amounts to be determined.

One Postdoctoral Fellowship awardee will be selected and will receive \$500 and a plaque. The winner of the Postdoctoral Fellowship Award will also be invited to serve on the CSS executive committee as the Postdoctoral Representative.

While abstracts may be submitted for multiple SOT awards, the CSS

awards will not be given to a student or postdoc receiving another award for the same abstract. Awards will be announced during the CSS meeting at the Annual Meeting.

The CSS Officers will invite the Dharm Singh and First Place Postdoctoral winners to join the CSS Executive Committee meeting which is held at the Annual Meeting in March.

## **CSS 2012 Student and Postdoctoral Awards**

The CSS Officers would like to congratulate the student and postdoctoral award winners for 2012 that were selected from a large pool of outstanding submissions. The Posters were presented at scheduled poster sessions AND during the CSS Reception in at the last annual meeting in San Francisco.

### **2012 Student Awards:**

**First Place/Dharm V. Singh Carcinogenesis Endowment Graduate Student Award (\$500):**

**Sara Nowinski (The University of Texas at Austin)**

*Mitochondrial Uncoupling Protein 3 (UCP3) Antagonizes Epidermal Tumor Promotion and Growth Signaling Transition.*



Sara Nowinski and Dharm V. Singh

### **Graduate Student Awards:**

Second Place (\$300) - **Anthony Apostoli**. Queen's University, Kingston, Ontario Canada.

The role of PPAR $\gamma$  in mammary secretory epithelial cells during DMBA-induced breast tumorigenesis.

Third Place (\$200) - **Nicholas Mastrandrea**. University of Arizona, Pentoxifylline decreases cyclin D1 through proteosomal degradation and arrests renal cancer cells in the G1 phase.

Fourth Place (\$100) - **Amanda Smolarek**. Rutgers University. NJ. Dietary administration of  $\gamma$ - and  $\delta$ -tocopherol inhibits mammary carcinogenesis.

**2012 Postdoctoral Award (\$500):**

**Gayathri Chadalapaka, Ph.D. (Texas A&M University, College Station)** *Colon cancer stem cell markers are modulated by Sp (specificity protein) transcription factors.*

**Science Spotlight:**

**Dharm V. Singh Carcinogenesis Endowment  
Graduate Student Award**

**Mitochondrial Uncoupling Protein 3 (UCP3) Antagonizes  
Epidermal Tumor Promotion and Growth Signaling.**

*By: Sara Nowinski*

*Advisor: Edward Mills, Ph.D.*

*The University of Texas at Austin*

Almost eight decades ago, Otto Warburg discovered that compared to normal tissues, nearly all malignant tumors exhibit increased glycolysis and decreased oxygen consumption. This general property is so pervasive that it is now used clinically to image malignancies by [ $F^{18}$ ] fluoro-deoxyglucose positron emission tomography (FDG-PET). In opposition to this malignant phenotype, mitochondrial uncoupling proteins dissipate the electrochemical proton gradient across the inner mitochondrial membrane. This increases oxygen consumption by driving futile respiration that is uncoupled from ATP synthesis. Our lab previously generated hemizygous mice that express a keratin 5 – uncoupling protein 3 (K5-UCP3) transgene in the basal epidermis in order to study the effects of UCP3 expression on carcinogenesis. In a paper recently published in *Oncogene*, we demonstrated that K5-UCP3 mice are completely protected from the formation of skin carcinomas in response to a two-stage (DMBA/TPA) chemical carcinogenesis regimen. To provide insight into the mechanism of UCP3 induced cancer resistance, we inter-

bred K5-UCP3 animals and v-Ha-Ras transgenic (Tg.AC) mice that express an oncogenic Ras gene, producing bigenic K5-UCP3/Tg.AC mice. While wild type Tg.AC mice form tumors in response to treatment with the tumor promoter 12-O-tetradecanoylphorbol-13-acetate (TPA) alone, K5-UCP3/Tg.AC animals are protected from TPA induced tumorigenesis, indicating that UCP3 likely inhibits tumor promotion. We believe UCP3 causes this effect by decreasing the signaling pathway response to the tumor promoter TPA. My ongoing research further examines the cellular and molecular mechanisms underlying UCP3 driven chemoprevention.

Basal keratinocyte proliferation is normal in K5-UCP3 mice, however, unlike wild type skin, K5-UCP3 epidermis fails to display a proliferative response to TPA treatment. 24 hours following TPA application, BrdU labels roughly 40% of the interfollicular, basal keratinocytes in wild type FVB/N mice, while K5-UCP3 mice did not show a significant response above baseline (~7% labeling). Excitingly, we discovered that this lack of proliferation coincides with the absence of Akt (PKB) phosphorylation on both Serine 473 and Threonine 308 four hours after TPA application. The loss of Akt activation also corresponds to decreased expression of cyclin D1 and cyclin A, as well as increased expression of the cell cycle inhibitory proteins p21<sup>cip1/waf1</sup> and p27<sup>kip1</sup> 18 hours after TPA treatment. Ongoing experiments are examining additional downstream mediators of Akt signaling to determine the importance of this effect in the many cell growth, survival, and proliferative pathways that Akt is known to regulate, including the transcription factor FOXO1a, as well as the tumor suppressor Pcd4. Unlike wild type, serum starved K5-UCP3 primary keratinocytes similarly lack Akt activation in response to EGF treatment, suggesting that this lack of proliferative response is not limited to TPA.

Akt activation is controlled by two kinases, phosphoinositide-dependent kinase 1 (PDK1) and mammalian target of rapamycin 2 (mTORC2), which phosphorylate Akt on Threonine 308 and Serine 473, respectively. Expression and activation of both PDK1 and mTORC2 is unchanged in K5-UCP3 epidermis, leading us to believe that these kinases are not responsible for the difference in Akt activation seen in K5-UCP3 skin. Several phosphatase enzymes provide negative regulation on Akt signaling, either by removing an activating phosphorylation (protein phosphatase 2A, PP2A, PH domain and leucine rich repeat protein phosphatase, PHLPP), or through decreasing Akt recruitment to the plasma membrane (Phosphatase and tensin homolog, PTEN). When treated with okadaic acid (OA), an inhibitor of PP2A, K5-UCP3 mice displayed a similar degree of Akt activation to that seen in wild type mice. This suggests that PP2A activity may be increased in K5-UCP3 epidermis, and that once PP2A is inhibited, then PDK1 is able to phosphorylate Akt comparably in both wild type and K5-UCP3 epidermis. PP2A is a redox

sensitive phosphatase that is inhibited by high levels of reactive oxygen species (ROS). Uncoupling proteins are well known for their ability to lower mitochondrial ROS production, and indeed, recent preliminary data has suggested that mitochondrial superoxide production is lower in K5-UCP3 primary keratinocytes (flow cytometry with dihydroethidium). We hypothesize that this lowering of cellular ROS may lead to the hyper-activation of PP2A, resulting in the observed blockade of Akt activation. Future experiments will focus on how UCP3 overexpression affects cellular redox homeostasis and PP2A activation, to mechanistically prevent Akt activation.

## ***First Place Postdoctoral Award***

**Colon cancer stem cell markers are modulated by Sp  
(specificity protein) transcription factors.**

*By: Gayathri Chadalapaka, Ph.D. Advisor: Stephen Safe, Ph.D.  
Texas A&M University*

Cancer is a leading cause of death worldwide and cancer mortality is projected to continue rising, with an estimated 12 million deaths in 2030. Among gastrointestinal cancers, colon cancer is the second most common and is the fourth leading cause of cancer related mortality, accounting for about 6% of all cancer-related mortality. Several studies show that colon cancer stem cells (CSCs), a small subset of cells within a tumor, are responsible for its malignancy, chemo-resistance and metastasis.

Previous studies indicate an the essential role for specificity protein (Sp) transcription factors Sp1, Sp3 and Sp4 as important therapeutic targets in colon cancer due to their over-expression in colon cancer cells and minimal expression in non-tumor tissue. Methyl 2-cyano-3,11-dioxo-18 $\beta$ -olean-1,12-dien-30-oate (CDODA-Me) is a synthetic derivative of glycyrrhetic acid, a triterpenoid found in licorice extracts and the anticancer activities of CDODA-Me and its effects on Sp protein expression in colon cancer cells as well as colon cancer stem cells were investigated in this project. 'HT29' colon cancer cell lines have been reported to possess subsets of cancer stem cell population called the 'sub-population', and these cells are used as a known candidate cancer stem cell line in this study. Apart from expressing stem cells markers CD44+/CD24+/ESA (epithelial specific antigen), HT29 cells also possess high drug efflux pumps that contribute to resistance against gemcitabine therapy. In the current study we have demonstrated that the cancer stem cell markers such as CD44 and CD24 are Sp modulated genes and Sp proteins contribute to colon cancer stem cell characteristics.

Preliminary data from this study suggests that anticancer activity of CDODA-Me, a glycyrrhetic acid derivative is due to Sp protein degradation and that CDODA-Me decreases cancer stem cell populations and expression of cancer stem cell markers through downregulation of Sp1, Sp3 and Sp4 transcription factors.

Results show that HT29 colon cancer cells form spheres which express several cancer stem-cell markers including increased expression of CD44, CD24 and aldehyde dehydrogenase (ALDH1). HT29 cells formed a significant number of primary and secondary spheres that are consistent with a population of cells enriched in colon cancer stem cells. Results also show that CDODA-Me decreased HT29 adherent cell survival, expression of Sp1, Sp3 and Sp4 proteins, and sphere formation. In the same cell line, CDODA-Me also decreased CD44 and ALDH1 expression that are cancer stem cell markers. Moreover, results of RNA interference studies show that the CD44 stem cell marker is also a Sp-regulated gene in HT29 cells. These results suggest that colon cancer stem cells also overexpress Sp1, Sp3 and Sp4 proteins that can be targeted by anticancer agents. The anticancer activity of CDODA-Me and Sp protein knockdown and effects on other novel cancer stem cell markers are currently being investigated. Future studies involve study of cancer stem cell enriched population growth as xenografts in athymic nude mice and the effect of CDODA-Me in reducing the tumor growth. The results from this study demonstrate that Sp transcription factors are overexpressed and functional in colon cancer stem cells and drugs such as CDODA-Me target this sub-population of cells.

### ***Help Make the 2014 Annual Meeting a Success!***

It is time to begin planning for the 2014 annual SOT meeting in Phoenix, Arizona. Program proposals for the 2014 meeting will be due soon (April 30, 2013). If you have suggestions for symposia, workshops, roundtables or CE courses, please contact Helmut Zarbl ([zarbl@eohsi.rutgers.edu](mailto:zarbl@eohsi.rutgers.edu)), Miriam Poirier ([poirierm@dc37a.nci.nih.gov](mailto:poirierm@dc37a.nci.nih.gov)), or Elaine Faustman ([faustman@u.washington.edu](mailto:faustman@u.washington.edu)). Alternatively, bring your suggestions to the 2013 Carcinogenesis mixer, so proposed topics for the 2014 sessions can be discussed during the annual CSS business meeting.