Carcinogenesis Specialty Section Newsletter

Society of Toxicology

In this Issue

President's Message

By: Vernon Walker

The SOT 2011 Annual Meeting in Washington, DC promises to be a stirring get-together due a strong scientific program plus activities marking the 50th Anniversary of the meeting. One of the ideas to celebrate SOT's first 50 years of success was the creation of a Time Capsule that will be opened by future leaders at the 75th SOT Annual Meeting. Each Specialty Section (along with Special Interest Groups and Regional Chapters) has been asked to provide small items representative of their group for inclusion in the Time Capsule. At the end of this Newsletter, there is an invitation soliciting your ideas for small items that the CSS might include, such as historical documents and momentos related to the founding of the CSS or interim activities by the CSS.

Charter members may recall that the CSS was founded in 1986 so that, while the SOT is having its 50th Anniversary Annual Meeting, the CSS will celebrate its 25th year as a specialty section (1986-2011). For those of you who do not know, the founding CSS officers included *Harold Grice* as President, *Hans Drobeck* as Vice President, *Theodore Farber* as Vice President-Elect, *Carl Shultz* as Secretary/Treasurer, and *Robert Kroes*, *Robert Squire*, and *Ching Wang* as Councilors. Given this timing, I am especially looking forward to seeing all of you at the CSS reception scheduled for Sunday evening March 6th from 6:30-8:00 PM where we will greet old friends and meet new ones over refreshments. At the reception, *Dr. James Swenberg* (member of the CSS Presidential track, 1987-1991) will offer a brief "tribute" to long-time members of the CSS. So, please attend and invite your colleagues to celebrate 25 years of the CSS.

At the reception, we will also discuss proposed continuing education, symposia, etc. for the 2012 meeting in San Francisco. A call for program proposals is included at the end of this Newsletter. The Scientific Program Committee and Continuing Education (CE) Committee pay special attention to suggestions by Annual Meeting attendees, and as a result have selected the CE 2012 Target Areas around which proposals may be developed, in addition to overall meeting themes soon to be announced. The CE Target Areas for 2012 include:

- *Assessment of the Role of Neuro-inflammation in Neurotoxicity*
- *Non-coding RNAs and Their Role in Biology and Toxicology*
- *Drug Metabolism*

Winners of the CSS student and postdoctoral awards also will be recognized at the reception. After the awards ceremony, please take time to congratulate the winners who will be available to show and discuss their posters on displayed boards at the reception. A listing of the awardees is included in this Newsletter, plus the work of two of the winners is highlighted below under "Science Spotlight".

It has been a pleasure serving you as the section president, and I would like to thank all of the members of the CCS who have participated in

various activities this year, including participation in the scientific program at the 50th SOT meeting. Last year we received an outstanding group of proposals from CSS members and the CSS has sponsored a great slate of symposia and workshops, as well as a CE course for the Washington, DC meeting (see the listing below in this Newsletter).

David Williams will be taking over as CSS President after the meeting in Washington, DC, and I wish to thank the current Officers of the CSS for their contributions throughout the year. I especially want to give thanks to Helmut for being the point of contact for nominations for this year's program, to Heather for her coordination and collection of the student and postdoctoral submissions for awards at the meeting, to Charlene for assisting with the preparation of the Annual Report and the budget guide estimates for CSS activities at the SOT meeting, to Ivan for handling of the plaques and checks for the CSS awards, and to David for being a sounding board and for organizing a slate for our next Board for 2011-2012.

2010-2011 CSS Officers and Student Representatives

President:	Vernon Walker (vwalker@uvm.edu)
Vice President:	David Williams (david.williams@oregonstate.edu)
Vice President-elect:	Helmut Zarbl (zarbl@eohsi.rutgers.edu)
Secretary/Treasurer:	Ivan Rusyn (ivan_rusyn@unc.edu)
Senior Councilor:	Heather Kleiner (hklein@lsuhsc.edu)
Councilor:	Nancy Doerrer (ndoerrer@hesiglobal.org)
Councilor:	Charlene McQueen* (mcqueen@auburn.edu)
SAC Representative:	Michael Borland (mgb149@psu.edu)

** Past President*

Important Dates

March 6, 2011	CSS Reception, Sunday Night 6:30-8:00 PM
March 7, 2011	CSS Officer's Breakfast, Monday Morning 6:30-7:45 AM
April 30, 2011	Proposal Outlines for SOT 2012 Symposia, Workshops, Roundtables, or CE Courses Due

Students and Postdocs: Join your peers and colleagues at the following events during the 2011 SOT Annual Meeting in Washington, DC. Check your program for location information. For more details of Student and Postdoctoral Scholar events, visit http://www.toxicology.org/ai/meet/am2011/studevent.asp

Event	Date	Time
Continuing Education Courses*	Sunday, 3/6	All day
Lunch with an Expert*	Sign up online: https://www.toxicology.org/ai/spd/lunchexpert.asp	
Student/Postdoctoral Scholar Mixer*	Sunday, 3/6	8:00-9:30 PM
Conversation with Dr. Collins*	Monday, 3/7	9:30-10:30 AM
In Vitro Toxicology Lecture and Luncheon for Students*	Monday, 3/7	NOON-1:20 PM
Postdoctoral Assembly Luncheon*	Tuesday, 3/8	NOON-1:15 PM
50th Anniversary Silent Auction	To be decided	

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

2011 CSS Student and Postdoctoral Awards

The CSS Officers would like to congratulate the student and postdoctoral award winners for 2011 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. These will be displayed at their scheduled poster sessions AND during the CSS Reception. Remember to encourage graduate students and postdoctoral fellows to submit their abstracts for consideration in 2012. To qualify, your work must be related to the field of carcinogenesis. The due date for submission will be around October-November 2011.

Dharm V. Singh Carcinogenesis Endowment Graduate Student Award ($500): **Jessica Graham (EOHSI / UMDNJ & Rutgers)**
The Fry Tumor Suppressor Encodes an Inhibitor of Epithelial Mesenchymal Transition

1st Prize ($500): **Graham Skelhorne-Gross (Queen's University)**
The In Vivo Rode of Adipoctye-Specific PPARγ in Environmental Risk Factor-Mediated Breast Tumorigenesis

2nd Prize ($300): **Satya Sreehari Pathi (Texas A&M University)**
GT-094, a NO-NSAID, Inhibits Colon Cancer Cell Growth by Activation of a Reactive Oxygen Species (ROS)-MicroRNA-27a:ZBTB10-Specificity Protein (Sp) Pathway

3rd Prize ($100): **Lauren Mordasky-Markell (Penn State University)**
Pharmacological Inhibition of the TGFβ1 Type I Receptor Induces Premalignant Keratinocyte Terminal Differentiation

Postdoc Award ($500): **Zhengyu Yin (NIEHS)**
RAP80 Plays a Critical Role in Maintaining Genomic Stability

2011 SOT Scientific Sessions Endorsed by the Carcinogenesis Specialty Section

Continuing Education:

- Epigenetics in Toxicology: Introduction, Mechanistic Understanding, and Applications in Safety Evaluation (*Sunday, March 6, 8:15 am – noon*)

Symposia:

- Epigenetics, Metals, and Cancer (*Monday, March 7, 2 pm – 4:45 pm*)
- Uncovering the Role of Non-Coding RNAs in Toxicology (*Tuesday, March 8, 9 am – 11:45 am*)
- Mechanisms of Inflammation in Skin Carcinogenesis (*Wednesday, March 9, 9 am – 11:45 am*)
- New Insights into the Nrf2-Keap1 Pathway and Its Impact on Human Disease (*Wednesday, March 9, 9 am – 11:45 am*)

Workshops:

- Disease Prevention: The Next 50 Years (*Monday, March 7, 9:15 am – noon*)
- New Approaches for Integrating of Toxicological and Epidemiological Data to Better Inform Risk Assessment (*Monday, March 7, 9:15 am – noon*)
- Using Mode of Action Data to Guide Quantitative Cancer Risk Assessment: A Case Study of Hexavalent Chromium in Drinking Water * (*Tuesday, March 8, 9 am – 11:45 am*)

** Primary Sponsor*

Science Spotlight:
Dharm V. Singh Carcinogenesis Endowment Graduate Student Award

The *Fry* tumor suppressor encodes an inhibitor of epithelial mesenchymal transition

By: Jessica Graham, Environmental and Occupational Health Sciences Institute, University of Medicine and Dentistry of New Jersey & Rutgers University. Advisor: Helmut Zarbl, PhD

Background. Laboratory rat strains vary significantly in their susceptibility to mammary carcinogenesis. Using a genetic backcross between the intermediately sensitive F344 strain (F344) and the resistant Copenhagen strain (Cop), we mapped a putative Mammary Carcinoma Susceptibility (Mcs) Locus to the long arm of rat chromosome 12. This locus is comprised of a 5.6 Mb region whose synteny is conserved on human chromosome 13, and was previously proposed to harbor the human BRCA3 gene. We therefore examined and compared the DNA sequences and expression levels of several candidate genes within the rat locus, including those previously implicated in various cancers. We identified two non-synonymous mutations in the rat ortholog of the *Drosophila furry* (*Fry*) gene in the susceptible F344 strain. Both of these mutations are in sequences of the gene that are invariant among all higher eukaryotes examined, suggesting that these amino acid substitutions impact Fry function. One of these polymorphisms substitutes a serine for an alanine residue at codon 2170 in the F344 strain, creating a *de novo* phosphorylation site within a concensus sequence that has a high probability (98%) of being a substrate for several protein kinases implicated in carcinogenesis. Although uncharacterized in mammalian cells, in the *Drosophila* the fry protein regulates the activity of the Tricornered family of kinases by serving as a scaffold protein that binds these kinases and their substrates. Fry also plays a role in epithelial cell proliferation, separation, morphogenesis and polarization. The human homologs of the Tricornered kinases are NDR kinases which were recently shown to possess tumor suppressor characteristics. These findings raise the possibility that the *FRY* gene regulates susceptibility to carcinogenesis by its effects on the activity of NDR kinases. Interestingly, pre-neoplastic lesions are induced in the mammary tissue in both the F344 and Cop strains treated with carcinogens, suggesting that Fry contributes to differential susceptibility by inhibiting progression of initiated cells to malignancy.

Hypothesis. Our data supports the hypothesis that polymorphisms in the F344 *Fry* homolog result in increased susceptibility to mammary carcinogenesis. Our

hypothesis is that **decreased expression, function or loss of mammalian FRY results in increased susceptibility to mammary carcinogenesis and progression of breast cancer.** The purpose of our studies is to test the hypothesis that the *FRY* gene encodes a mammary carcinoma susceptibility gene, and to define the molecular mechanisms through which FRY modulates breast cancer susceptibility. The results of these studies are expected to enable the future development of novel approaches to breast cancer screening, diagnosis, prevention, and treatment.

Significance. One in eight women in the United States will be diagnosed with breast cancer, which remains the leading cause of cancer death in women. Genetic testing has permitted women from high risk breast cancer families with the options of determining if they are at increased risk for breast cancer, and pursuing chemopreventive or surgical interventions to reduce their risk of cancer incidence and/or mortality. However, mutations in BRCA 1 and 2 genes are implicated in less than 10% all of breast cancers and are present in only a fraction of familial breast cancers. These statistics indicate that not all breast cancer susceptibility genes can be identified by linkage analysis in high risk families. As an alternative, we used linkage analysis in inbred rat strains and identified the *FRY* gene as a putative tumor suppressor. Consistent with its function as a putative tumor suppressor, our research demonstrated that *FRY* gene expression was decreased in a majority of rat mammary tumors, as well as all human breast carcinoma cell lines examined.

To evaluate the function of the *Fry* gene, we stably transfected the triple-negative, human MDA-MB-231 mammary tumor cell line with the wild type (wt) allele from Cop (231wCFry). Ectopic expression of the wild-type Cop allele resulted in decreased tumor growth and diminished invasiveness of the triple-negative breast tumor cell line *in vivo*. We also analyzed the top 691 genes which were upregulated or downregulated ten-fold or greater in 231wCFry relative to MDA-MB-231. Significantly, the major molecular and cellular functions represented by this gene set involve cell growth, cell development and cellular and tissue organization. Also, the top five diseases and disorders represented by this gene set included cancer, genetic disorder and neurological disease. Interestingly, the Wnt/β-catenin signaling pathway, which maintains cell polarization, was the canonical pathway most significantly altered by ectopic *Fry*. Using immunocytochemistry on cell colonies grown in Matrigel, we found that in 231wCFry cells the levels and subcellular distribution of β-catenin and α4-integrin, both markers of epithelial cell differentiation, more approximated those in the nontumorigenic MCF10A human mammary epithelial cell line than those in MDA-MB-231, suggesting *Fry* restored a differentiated phenotype to the cancer cells *in vitro*. Based on this pathway analysis, we hypothesized that FRY is

involved in multiple pathways and plays an important role in the growth and development of human mammary epithelial cells.
To determine how *FRY* gene expression may be related to human breast cancers, we utilized human microarray data available in the Oncomine 3.0 Cancer Profiling Database (http://www.oncomine.org/main/index.jsp). Interestingly, in nine studies that reported tumor grade along with expression data (Grade 1: 321, Grade 2: 784, Grade 3: 626), *FRY* expression was found to be decreased in Elston Grade 3 tumors relative to Elston Grade 1 tumors at the $p<0.0001$ level of significance. Our results suggest that decreased *FRY* expression or loss is associated with the least differentiated and most aggressive grade of tumor types.

These studies support the hypothesis that the mammalian *FRY* gene encodes a tumor suppressor gene and they provide insight into its molecular mechanism of action in mammary epithelial cells. Moreover, we have demonstrated that human *FRY* is decreased in poorly differentiated mammary tumors relative to well differentiated mammary tumors, indicating that *FRY* plays a role in human breast cancer progression. The results from our research have the potential to be used in population based studies to assess the role of *FRY* mutation in human breast cancer and develop new genetic tests to screen for increased cancer risk. Mechanistic insights into *FRY* mediated tumor suppression will provide insights into gene-environment interactions that increase the penetrance of mutant *FRY* alleles, and enable the development of mechanistically based preventive interventions and therapeutics.

Science Spotlight: First Place Postdoctoral Submission

RAP80 Plays a Critical Role in Maintaining Genomic Stability

By: Zhengu Yin, The National Institute of Environmental Health Sciences. Advisor: Anton Jetten, PhD

Abstract. The DNA damage response (DDR) coordinates activation of cell cycle checkpoints, apoptosis and DNA repair networks to ensure accurate repair and genomic integrity. Phosphorylation of the histone H2A variant, referred as γ-H2AX, is one of the initial signaling events which sense DNA double strand breaks and is required for the subsequent recruitment of many DDR proteins to sites of DNA damage. Upon DNA damage events like ionizing irradiation (IR), ubiquitin interaction motif (UIM)-containing protein RAP80 binds to poly-ubiquitin chain of H2A and γ-H2AX, and mediates DNA repair events by recruiting DDR mediators and effectors, especially BRCA1. In this study, RAP80 knockout (KO) mice were generated and characterized. In contrast to the embryonic lethal phenotype in BRCA1 KO mice, RAP80 KO mice are viable and there appears to be no major anatomic defect, which suggests RAP80 might be involved in DDR signaling pathways independent of BRCA1. Nevertheless, mouse embryonic fibroblasts (MEFs) from RAP80 KO mice exhibited slower proliferation as well as higher percentage of premature senescence compared to wild type (WT) MEFs. RAP80 KO MEFs also showed increased spontaneous and IR-induced genomic instability, which led to prolonged G2/M cell cycle arrest. There is higher percentage of spontaneous γ-H2AX positive cells in RAP80 KO MEFs compared to WT MEFs; IR induces more nuclear fragmentation in RAP80 deficient MEFs than WT controls. Loss of RAP80 increased sensitivity to IR both in vivo and in vitro, which is consistent with the finding that loss of RAP80 activated p53, which, following DNA damage, results in an increased transactivation of several p53 target pro-apoptotic genes. Preliminary tumorigenesis studies suggested that RAP80 KO mice exhibit an increased susceptibility to the spontaneous development of lymphoma and the development of DMBA-induced mammary gland cancer. Altogether, these data indicate that RAP80 functions as a tumor suppressor gene and deficiency of RAP80 leads to genomic instability and predisposition to cancer.

RAP80 is a UIM-containing protein playing a critical role in DNA repair. In previous studies, we and other groups demonstrated that RAP80 is part of a BRCA1-BARD1-ccdc98 (Abraxas) complex and promotes the translocation of these complexes to DNA damage sites and as such is involved in DSB repair and cell cycle checkpoint control following IR exposure. We also reported that RAP80

interact directly with ERalpha and positively modulate ERalpha-mediated transactivation. Interesting, we then identified that RAP80 can function in an autoregulatory loop consisting of RAP80, HDM2, and the p53 master regulatory network. Although it is inducible by p53, RAP80 is able to regulate p53 through an association with both p53 and the E3 ubiquitin ligase HDM2, providing HDM2-dependent enhancement of p53 polyubiquitination. Depletion of RAP80 by small interfering RNA stabilizes p53, which, following DNA damage, results in an increased transactivation of several p53 target genes as well as greater apoptosis. These imply an important role for this loop in genome stability and oncogenesis.

Mouse models deficient of DNA repair genes. *Atm*, *H2ax*, *Mdc1 and Rnf8* are the genes upstream of RAP80 in terms of DDR mediated by phosphorylation and ubiquitination of H2A and H2AX. Notably, mouse models for mutations/deletion of *Atm*, *H2ax*, *Mdc1*, *53bp1* and *Rnf8* have demonstrated the roles of these signaling proteins in various processes including development, meiosis, class switch recombination, genomic integrity, and spontaneous cancer development. For example, Rnf8 deficiency leads to increased sensitivity to ionizing radiation both in vitro and in vivo. In addition, Rnf8-/- mice exhibit increased genomic instability and elevated risks for tumorigenesis. Although BRCA1 deficient mice demonstrate somewhat distinct phenotype such as embryonic lethality accompanied by widespread apoptosis, elimination of one Trp53 allele completely rescues this embryonic lethality and Brca1-/- Trp53 +/- mice are prone to tumor development. *In vitro* data showed that knockdown cell lines for RAP80 are unable to form or retain IRIF for BRCA1 and other downstream DDR proteins. These cells also exhibit increased radiosensitivity and impaired cell cycle checkpoints.

The above information and our previous studies in our laboratory provide a compelling basis for our overall hypothesis that **RAP80 is a tumor suppressor gene and deficiency of RAP80 leads to genomic instability and predisposition to cancer**. Some of the critical questions that need to be addressed are the following: What is the phenotype of RAP80 deficient mice? Does loss of RAP80 lead to genomic instability and radiosensitivity? Are RAP80 deficient mice prone to spontaneous or chemical-induced tumor development? What kind of tumor will they develop? The objectives of this work are highly significant since 1) they focus on human diseases based genetic deficiency that are of real and relevant human interest, 2) they will test a particularly novel hypothesis for the actions of the critical player in DDR and tumor suppressing, 3) they will add to our knowledge and understanding of the signaling pathways involved in DNA repair, 4) they will assist in our understanding of how these components may prevent cancer and/or progression, and 5) they will offer avenues for new and novel therapeutic approaches for radiotherapy and other anticancer procedures.

Action Required!

SOT 50th Anniversary Celebration and TIME CAPSULE

SOT leadership has requested that each specialty section provide small items representative of their group for inclusion in the Time Capsule that will be opened by future leaders at the 75th SOT Annual Meeting. Possible small items for inclusion by CSS include historical documents, momentos, or current state-of-the-art materials (nonhazardous).

It has been requested that the size of items be limited to less than 12" x 12". Please send your ideas for appropriate items for the CSS to include in the Time Capsule, including historical artifacts from earlier CSS activities that come to mind or that may be in your possession. For making suggestions, please contact Vernon Walker (vwalker@uvm.edu), David Williams (david.williams@oregonstate.edu), or Helmut Zarbl (zarbl@eohsi.rutgers.edu).

Help Make the 2012 Annual SOT Meeting a Success!

It is time to begin planning for the 2012 annual SOT meeting in San Francisco, CA. Program proposals for the 2012 meeting will be due soon (April 30, 2011). If you have suggestions for symposia, workshops, roundtables or CE courses, please contact Vernon Walker (vwalker@uvm.edu), David Williams (david.williams@oregonstate.edu), or Helmut Zarbl (zarbl@eohsi.rutgers.edu). Alternatively, bring your suggestions to the Carcinogenesis mixer, where proposed topics for next year's sessions can be discussed during the business meeting.