THE DOSSIER

The Digest on CCR Staff Scientists and Staff Clinicians: Information, Employment and Research

March 2015 Issue 19

From the Editor

Welcome to the March issue of The Dossier, a newsletter dedicated to the Staff Scientists and Staff Clinicians (SSSC) of the CCR!

This issue contains important messages from the Director's Office, a special article by Yamini Dalal, Ph.D., and information on the 2015 SSSC Retreat by Anu Puri, Ph.D. and Sergey Tarasov, Ph.D. We feature Vladamir Majerciak, Ph.D., in our SSSC Corner, while Shree Ram Singh, Ph.D., and Stephen Lockett, Ph.D., describe their collaborative efforts at the Optical Microscopy and Analysis Laboratory (OMAL) Resource. In addition, the published work of Michael Nickerson, Ph.D., is highlighted in our Author's Corner.

We hope to continue to provide pertinent information to aid in the success of SSSCs. Please send your contributions, suggestions, and comments to budhua@mail.nih.gov.

Anuradha Budhu, Ph.D. (SS)
Editor-in-Chief
Laboratory of Human Carcinogenesis

In This Issue

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From the Office of the Director

CCR Clinical Program All Hands Meeting

Highlights and Initiatives

I'm pleased to update you on some important initiatives and successes in the CCR Clinical Program. I am delighted to report that we have created new professional tiers for our Staff Clinicians, similar to those available to Staff Scientists. It has long been recognized that NIH Staff Clinicians, who have a wide range of roles and skills and are vital to the success of clinical research, deserve a "career ladder" that recognizes their value and accomplishments. While the NIH has not yet come to agreement on this, institutes are permitted now to develop their own tier structure. You can read more about the new tiers in this edition. Also in this edition you'll read about our efforts to continue to improve our collection and management of human biospecimens. This is a very important issue as was brought home by the "clean sweep" project following the Ebola outbreak that has been garnering national headlines. Many of you will no doubt play a part of ensuring that we are managing our human specimens with the integrity each patient deserves and I thank you in advance for your assistance.

I hope many of you attended the "All Hands" Meeting for clinical senior staff held on December 8, 2015. At that meeting we updated the community about newly appointed senior staff, awards, recent scientific advances from the clinical program, the CCR FLEX initiative, results of an evaluation of the recently-established Office of Research Nursing, and improvements to CCR protocol scientific review process. The protocol scientific review process is still nascent, but I'm confident that it is making our research better and encouraging collaboration. Our goal has been to establish a constructive, community-driven process that will result in better clinical protocols. To do that, we need our community to become engaged. CCR senior staff (Principal Investigator, Senior Scientist/ Clinician, Staff Scientist, Staff Clinician, and Assistant Clinical Investigator) are eligible to vote at these meetings. I urge Staff Clinicians and Staff Scientists interested in translational research to attend these meetings and be part of this important process. The CCR concept reviews are held the first and third Friday of each month in the Lipsett Auditorium, Building 10 from 1:30-3:30 p.m. Join the listserv for notifications: https://ccrod.cancer.gov/confluence/display/CCRCRO/CCR+Scientific+Review. Another "All Hands" meeting is planned for May 27, 2015, 2-3PM in the Lipsett Auditorium, Building 10. I hope to see you there.

Lee J. Helman, M.D.
Scientific Director for Clinical Research

The OD Corner

CCR Announces Staff Clinician Professional Tiers

The CCR announced in February that it has created two new position tiers for Staff Clinicians: "Associate Research Physician" and "Senior Research Physician." These tiers are similar to those available to NIH Staff Scientists. They are intended to recognize the accomplishments of CCR Staff Clinicians, who play important roles, have unique skills, and make significant contributions to the success of the CCR clinical research program. While the official NIH Intramural Professional Designation (IPD) will remain "Staff Clinician", these alternative CCR designations may be used in professional correspondence, websites, and CVs. Advancement to either tier will require formal endorsement by the Scientific Director for Clinical Research. Requests may be made through the quadrennial review process or ad hoc.

Associate Research Physician

Staff Clinicians approved for the tier, "Associate Research Physician", generally will have at least four years of post-fellowship experience. At this level, the physician is expected to have taken on leadership roles in the CCR; be knowledgeable in the development and conduct of clinical research trials, as demonstrated through a successful track record of implementing trials and/or completed training in areas related to human subjects research; and be active in the professional community. This position is envisioned as equivalent to an Associate Professor in the academic clinical educator track.

The following factors will be considered:

- Must have received an "Outstanding" rating from most recent CCR Quadrennial Review;
- Must play a major support role within a quality clinical research program;
- Should deliver quality patient care to protocol participants as an Attending Physician and/or through a consult service;
- Should have an excellent knowledge of the conduct of human subject research trials;
- Should have contributed to development and execution of quality clinical research protocols;
- Should have served in leadership role(s) in CCR/NCI and/or NIH (e.g., CCR Protocol Concept Reviewer, service on CCR national search committees);
- Should be involved in professional community activities such as national meetings, professional organizations, and extramural collaborations;
- Should have demonstrated a commitment to training and mentoring of clinical staff;
- May have received NIH and/or NCI award(s)

Senior Research Physician

Staff Clinicians approved for the tier, "Senior Research Physician", will be considered national or international leaders in their field. At this level, physicians are expected to be active leaders in CCR (e.g. CCR Advisory Board, CCR Protocol Concept Review chairperson, service on CCR national search committees, branch Fellowship Program Director/ Assistant Director); develop and serve as PI of one or more clinical research trials; and actively mentor other clinical staff. These individuals would have stature such that they are called upon as experts by outside institutions, are invited to give seminars at research institutions and national meetings, hold important roles in professional organizations, and/or serve on grant study sections. This position is envisioned as equivalent to that of a Full Professor in the academic clinical educator track.

In addition to the factors considered for Associate Research Physician as listed above, the following additional factors will be considered for advancement to this level:

- Must have received an "Outstanding" rating on the last two most recent Quad Reviews;

CCR Announces Staff Clinician Professional Tiers Con't

- Must be seen as an expert in the field, held in high regard by peers, as evidenced by such factors as being consulted by others inside and outside of NIH, invitations to speak at important professional meetings, receipt of national/international awards, and/or leadership role(s) outside of NIH in extramural community, and/or quality clinical research publications.

CCR OD

New Human Biospecimens Must Be Electronically Tracked in CCR by March 1, 2015

On January 14, Dr. Lee Helman, Scientific Director for Clinical Research, announced his expectation that all new human biological samples obtained for research use (either obtained from patients at the Clinical Center or acquired from extramural sources) must be logged into a computerized software tracking system and be bar coded. This is necessary to comply with legal statutes and HHS policy. In a 2013 report, "Guidelines for Human Biospecimen Storage and Tracking Within NIH (http://sourcebook.od.nih.gov/oversight/BiospecimenGuidelines.pdf)," Dr. Michael Gottesman, Deputy Director for Intramural Research, noted:

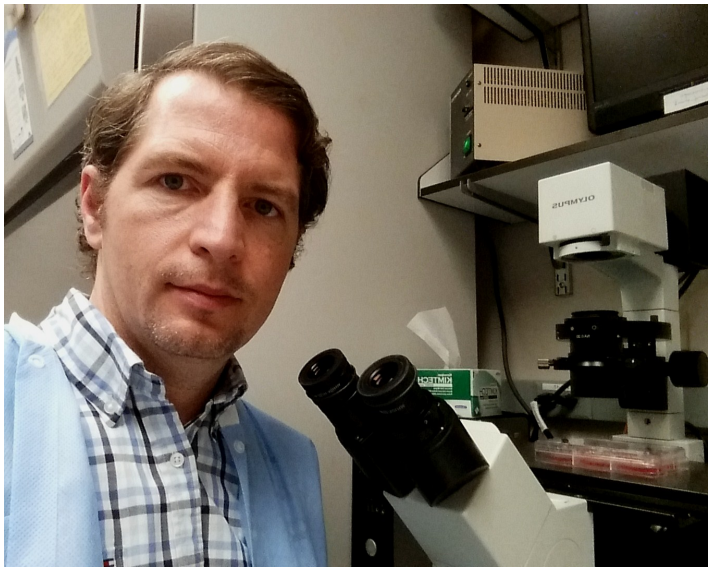
> *Biological specimens (or biospecimens") from study participants must be handled according to the highest ethical and scientific standards to maintain the public's trust, to preserve and protect the specimens and the substantial investment these resources represent, and to facilitate research by maximizing use of the specimens."*

There are four options for electronic tracking of human biospecimens in CCR: The Blood Processing Core (BPC), in the Office of the Clinical Director (Contact: Dr. Doug Figg); Labmatrix, a web-based CCR-supported system that is and can be used by any laboratory/branch (Contact: Dr. Jason Levine, CCR Office of Information Technology); Fisher Bioservices, at NCI's Frederick campus; and compatible existing lab/branch systems, provided such systems are able to communicate and transfer data quickly to a CCR platform. Existing, legacy samples do not need to be bar-coded by March 1. However, CCR will be requesting that such samples be back-cataloged in the future. CCR contacts for this requirement are Drs. Jason Levine and Doug Figg, who may be contacted with specific questions.

CCR OD

The SSSC Corner

Section Editor: Takashi Furukawa, Ph.D. (*SS*)



I received my Ph.D. training at the Institute of Virology, Slovak Academy of Sciences in Bratislava, one of the oldest institutes dedicated purely to basic virology research. Since its establishment in 1953, the institute strived to build close scientific connections with the NIH, as almost all of my colleagues working there either visited, trained, or collaborated with the agency. Therefore, by the time I graduated, I was very familiar with NIH research, and it was quite a natural choice to seek postdoctoral training there. During my Ph.D., I studied the virulence factors responsible for an increased oncogenic potential of newly appearing strains of Marek's disease virus, an animal herpesvirus causing a rapid onset of T cell lymphomas in domestic chickens.

For my postdoctoral training, I wanted to continue exploring the role of viruses in cancer development, so I was happy to find a position in the newly established lab of Zhi-Ming Zheng, M.D., Ph.D., focusing on RNA biology of oncogenic viruses. After joining his laboratory in 2003, I decided to pursue research focusing on Kaposi's sarcoma-associated herpesvirus (KSHV), another oncogenic herpesvirus linked to Kaposi's sarcoma and several atypical B cell lymphomas highly prevalent in AIDS patients. At that time, KSHV was a recently discovered virus, and we were just begining to understand its biology and role in cancer development. Our early work included an annotation of viral genes, mapping of viral transcripts and characterization of kinetics of viral genes expression.

At the same time we also began a study of KSHV ORF57, an essential viral protein involved in post-transcriptional regulation of KSHV gene expression. Despite its original description as a homologue for well-characterized herpes simplex virus type 1 ICP27 protein, we soon uncovered the profound functional differences between ORF57 and ICP27. For example, we described ORF57 as a novel splicing factor which is required for splicing of viral transcripts with suboptimal introns that is in contrast to ICP27 inhibitory effect on RNA splicing. Over several years, we also identified other important roles of ORF57 such as stabilization of viral transcripts and promotion of viral translation by counteracting miRNA-mediated pathway, and we identified numerous cellular co-factors required for each ORF57 function. These findings significantly extended our understanding of an essential role of ORF57 in the KSHV life cycle.

In 2011, I accepted a Staff Scientist position in Dr. Zheng's lab and began to focus my knowledge and expertise on more complex and challenging projects in the lab. As a result, we recently defined a genome-wide landscape of polyadenylation of all KSHV transcripts using a novel PA-seq technique, developed in collaboration Jun Zhu, Ph.D., from the DNA Sequencing and Genomics Core, NHLBI. Currently, we are continuing our research efforts to better understand how KSHV infection affects overall RNA biology in host cells. Beside my direct participation in research, I also enjoy the opportunity to train a new generation of postdoctoral and research fellows and to share my knowledge and personal experience at NIH on how to be a successful scientist in their future endeavors.

As a DC resident, I like to spend my free time with my friends enjoying the city life, including the vast array of museums, concerts, shows, dining, and other cultural activities. I enjoy meeting new people, as DC is

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Section Editor: Takashi Furukawa, Ph.D. (*SS*)

Dr. Majerciak is pictured enjoying a ski trip at Whitetail Resort in Pennsylvania.

a unique place where people from every country in the world are an integral part of everyday life. During the weekends, I take trips to surrounding mountains and beaches to escape the city and relax.

Vladimir Majerciak, Ph.D. *(SS)*
Tumor Virus RNA Biology Section
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The Core Corner

Section Editor: Anne Gegonne, Ph.D. (*SS*)

Drosophila In Vivo Model System to Study the Molecular Mechanisms Regulating Stem Cell Behavior and Tumorigenesis

In the Stem Cell Regulation and Animal Aging Section of the Basic Research Laboratory, headed by Steven Hou, Ph.D., we focus on understanding the molecular and genetic mechanisms by which stem cells regulate tissue homeostasis, regeneration, and tumorigenesis in *Drosophila* and mouse model systems. Stem cells have the tremendous ability to self-renew and can produce diverse types of differentiated cells. Stem cells hold great potential in developing novel cell-based therapies for several diseases, including cancer. A proper balance between stem cell proliferation and differentiation should be maintained to avoid tumor formation and degeneration of organ or tissues.

In 2003, when I started my postdoctoral training under the mentorship of Dr. Hou, I was not familiar with confocal microscopy techniques and stem cell biology. I came with a background in evolutionary and behavior genetics. At that time, Stephen Lockett, Ph.D., Director of Optical Microscopy and Analysis Laboratory (OMAL), NCI at Frederick, had the only core facility that provided confocal training and guidance to complete projects involving cell biology imaging. He generously provided confocal training and imaged our samples. Within a few months of training, we were able to capture high-resolution imaging of our samples independently. Dr. Lockett has always been very helpful and provided all technical details needed to succeed in our projects. Over the years, several updated versions of confocal microscopy have been released. With each new version, Dr. Lockett and his staff have provided training and help in finishing our projects. With the help of this core facility, we have published several high quality manuscripts.

Drosophila germline stem cells (GSCs) and their niches were identified before I joined the Hou Lab, however, interactions between stem cells and their niches were not clear. Using this high quality confocal facility at NCI at Frederick, which is essential for our

projects, in the last few years, we identified and characterized several new stem cells in adult *Drosophila* tissues including the kidney and gastrointestinal system. Most of our studies involved the *in vivo* characterization of stem cells in these organs (Figure 1A-F). During a genetic screen for genes that interact with the JAK-Stat signaling in male GSCs, we discovered that RapGEF/Rap signaling regulates stem cell anchoring to the niche by regulating E-cadherin-mediated cell adhesion. Furthermore, we have analyzed a novel tumor suppressor gene, *BHD* (Birt Hogg Dube syndrome) in *Drosophila* and demonstrated that *BHD* regulates GSCs maintenance by interacting with JAK-STAT and Dpp/BMP signaling pathways. Furthermore, we demonstrated that a single JAK-STAT signal from the niche orchestrates the competitive and dependent co-existence of germline

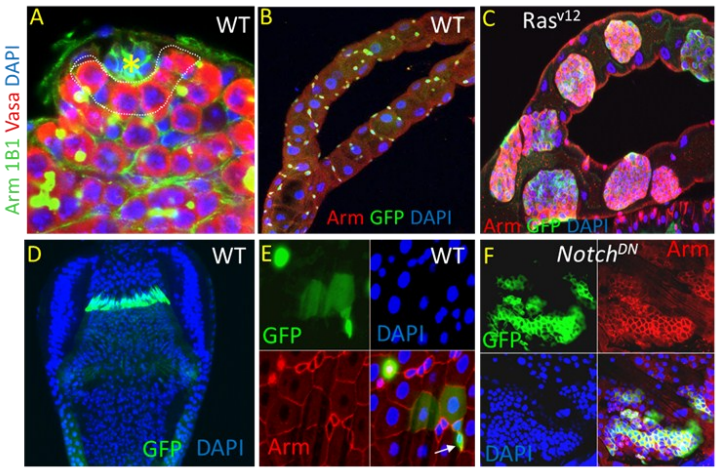


***Figure 1. In Vivo* labeling of germline and gastrointestinal stem cells in *Drosophila*.** (A) *Drosophila* male germline stem cells in adult testis. Vasa (red) marks germ cells including germline stem cells (GSCs-dotted line), Arm (β-catenin-green-membrane) marks the hub cells (niche, asterisk), 1B1 (adducin-related protein) in green dots and branched, marks the spectrosomes and fusomes. (B) *Drosophila* adult renal and nephric stem cells (RNSCs) marked by STAT-GFP (Singh *et al.* 2007). (C) Expression of a constitutively activated form of Ras transforms RNSCs into stem cell tumors (Zeng *et al.* 2010). (D) Gastric stem cells in *Drosophila* mark by Stat-GFP (Singh *et al.* 2011). (E) Midgut (small intestine) stem cells (ISCs-arrow) in adult *Drosophila*. (F) Knockdown of Notch transforms ISCs into stem cell tumors (Singh *et al.* 2012).

and somatic stem cells in the *Drosophila* testis niche. Over the years, we performed screens to identify JAK-Stat signaling interacting partners in the *Drosophila* stem cell system. Using a GFP reporter (Stat-GFP) for the JAK-Stat signaling, we found that signaling is activated in stem cells in testis, kidney, and gastrointestinal tissues. Therefore, Stat-GFP reporter can be used as a reliable stem cell marker. Based on lineage tracing and molecular marker labeling, we identified multipotent renal and nephric stem cells (RNSCs) in the *Drosophila* (Fig.1B) and demonstrated that an autocrine JAK/STAT signal regulates the RNSCs self-renewal. The adult fly kidney stem cells are relatively quiescent. However, we demonstrated that the activation of the JAK-STAT signal transduction pathway or the expression of the activated form of the *Ras* oncogene can transform RNSCs into stem cell tumors (Fig.1C). Using the Stat-GFP reporter, we also recently identified gastric stem cells (GaSCs) in the adult *Drosophila* (Fig.1D) and found that JAK-STAT signaling regulates GaSCs proliferation, wingless signaling regulates GaSCs self-renewal, and hedgehog signaling regulates GaSCs differentiation.

In the last few years, we have also completed genome-wide RNAi screens in the *Drosophila* gastrointestinal and male GSC system and identified a number of unique genes whose knockdown influences diverse aspects of stem cell properties. We are currently focused on characterizing some of the identified genes that regulate stem cell self-renewal, proliferation, and stem cell survival. Understanding the molecular mechanisms governing stem cell self-renewal or differentiation *in vivo* is not only crucial to using stem cells for future regenerative medicine and gene therapy, but it also will increase our understanding of the mechanisms underlying cancer formation, aging, and degenerative diseases.

References:

1. **Singh, S.R.**, Liu, W., and S.X. Hou. The adult *Drosophila* malpighian tubules are maintained by multipotent stem cells. *Cell Stem Cell,* 1: 191–203, 2007.

2. **Singh, S.R.**, Zhen, W., Zheng, Z., Wang, H., Oh, S.W., Liu W., Zbar B., Schmidt, L.S., and S.X. Hou. The *Drosophila* homologue of the human tumor suppressor gene BHD interacts with the JAK-STAT and Dpp signaling pathways in regulating male germline stem cell maintenance. *Oncogene,* 25:5933–5941, 2006.

3. Zeng, X., Han, L., **Singh, S.R.**, Liu, H., Neumüller, R.A., Yan, D., Hu, Y., Liu, Y., Liu, W., Lin, X., and S.X. Hou. Genome-wide RNAi screen identifies networks involved in intestinal stem cell regulation in *Drosophila. Cell Reports,* 10:1226-1238, 2015.

4. **Singh, S.R.**, Mishra, M.K., Kango-Singh, M. and S.X. Hou. Generation and staining of intestinal stem cell lineage in adult midgut. *Methods in Molecular Biology* 879: 47–69, 2012.

5. **Singh, S.R.**, Zeng, X., Zheng, Z. and S.X. Hou. The adult *Drosophila* gastric and stomach organs are maintained by a multipotent stem cell pool resides at the foregut/midgut junction in the cardia (Proventriculus). *Cell Cycle,* 10 (7), 1109–1120, 2011.

References Con't:

6. **Singh, S.R.**, Zheng, Z., Wang, H., Oh, S.W., Chen, X., and S.X. Hou. Competitiveness for the niche and mutual dependence of the germline and somatic stem cells in the *Drosophila* testis are regulated by the JAK/STAT signaling. *Journal of Cellular Physiology,* 223:500–510, 2010.

7. Zeng, X., **Singh, S.R.**, Hou, D., and S.X. Hou (2010). Tumor suppressors Sav/Scrib and oncogene Ras regulate stem cell transformation in adult *Drosophila* malpighian tubules. *Journal of Cellular Physiology*, 224:766-74, 2010.

8. Wang, H., **Singh, S.R.**, Zheng, Z., Oh, S.W., Chen, X., Edwards, K., and S.X. Hou. Rap-GEF signaling controls stem cell anchoring to their niche through regulating DE-cadherin-mediated cell adhesion in the *Drosophila* testis. *Developmental Cell,* 10: 117–126, 2006.

Shree Ram Singh, Ph.D. (SS)
Stem Cell Regulation and Animal Aging Section
Basic Research Laboratory

Optical Microscopy and Analysis Laboratory (OMAL) Resource

The OMAL Resource https://confocal.cancer.gov/cores/optical-microscopy-and-analysis-laboratory is an open, shared user facility. CCR scientists receive training from OMAL staff members to perform their own experiments using eight confocal and super-resolution microscopes for imaging living and fixed cells and tissues. Along with offering 3D fluorescence microscopy, OMAL provides cutting-edge computational expertise for visualization and extraction of quantitative information from images. The OMAL undertakes several microscopy development projects to sustain state-of-the-art microscopy capabilities for the NCI, and works collaboratively with CCR scientists on cancer and HIV biology research problems that push the limits of optical microscopy. In 2012 and 2013, OMAL scientists were authors on 16 publications and were acknowledged on 16 additional publications. OMAL strongly encourages scientists with potential needs for optical microscopy to meet us to discuss their research needs. The scientists in OMAL are Stephen Lockett, Valentin Magidson, Jiji Chen, De Chen, Kaustav Nandy, Prabhakar Gudla and Kimberly Peifley.

Stephen Lockett, Ph.D.
Director, Optical Microscopy and
Analysis Laboratory (OMAL)

The SSSC Retreat

The National Cancer Institute's Center for Cancer Research (CCR), Division of Cancer Epidemiology and Genetics (DCEG), and the Frederick National Laboratory for Cancer Research (FNLCR) will hold the annual Staff Scientist and Staff Clinician retreat on May 1st, 2015 at NCI Shady Grove.

The last two retreats included interactive forums that were very successful, and we are optimistic that this year will not be an exception. The 2015 retreat's theme is "From Cancer Genomics to Therapeutics". The forum will start with a keynote presentation by Prof. Raghu Kalluri, M.D., Ph.D., (Chair of the Department of Cancer Biology, MD Anderson Cancer Centers). Dr. Kalluri's pioneering work on exosomes has uncovered the mechanisms by which tumor cells can transmit transforming mRNA and miRNA to non-tumor-forming cells and thus promote tumor growth.

Dr. Kalluri's presentation will be followed by short visionary talks by the top experts in the field of cancer genomics: Stephen J. Chanock, M.D., Director, Division of Cancer Epidemiology and Genetics, Louis Staudt, M.D., Ph.D., Co-Chief of the Lymphoid Malignancies Branch and Head of the Molecular Biology of Lymphoid Malignancies Section, CCR; Javed Khan, M.D., Deputy Chief of the Genetics Branch and Head of the Oncogenomics Section, CCR and Michael Dean, Ph.D., Deputy Director of the Cancer and Inflammation Program and Head of the Human Genomics Section, CCR. The discussion topics will include, but will not be limited to the following questions:

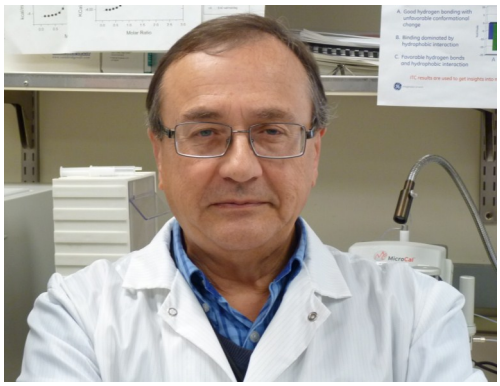
●Does the absence of drugs targeting non-druggable drivers impede the use of genetic analysis in the clinics? ●Are genome wide association studies worth the effort? ●Does tumor heterogeneity preclude the effective use of genetic analysis in the clinics? ●How important is "genomic junk", the non-coding genome?

The panel discussion is designed to be highly interactive, informative, and productive. We expect very lively conversations and questions and urge our fellow SSSC to be active in these discussions. So bring your burning questions or just ask them on the spot to make the retreat even more exciting. We believe that this session will lead to new insights and ideas for all of us.

As always, we invite you to submit abstracts and participate in poster sessions in one of three categories: Clinical and Translational Research; Basic Research; Technological and Methodological Development. Travel awards will be given to the top three posters presented at the retreat. The poster session, a highlight of the retreat, provides a unique opportunity to network with colleagues, so we strongly encourage you to participate. Again this year. SSSCs who submit the best abstracts will be invited to give oral presentations about their work. We also will discuss several topics, related to professional development, quadrennial review and other important aspects of the SSSC Organization's activities.

So please mark the date on your calendars – May 1, 2015, consider submitting an abstract and be ready! Look for details for online registration and abstract through the SSSC Listserv and announcements throughout the NCI. We look forward to seeing you at the retreat.

Anu Puri, Ph.D. (SS)
and Sergey Tarasov, Ph.D. (SS, Facility Head)
2015 SSSC Retreat Co-Chairs



The PI Corner

Section Editor: Lakshmi Balagopalan, Ph.D. (*SS*)

The role of the Staff Scientist has fast become an attractive and competitive option in the academic world. Of my 2007 postdoctoral fellows at the Fred Hutchinson Cancer Research Center, all of whom trained in elite labs and published papers in widely disseminated journals, the majority are now Staff Scientists. These talented scientists chose not to pursue the traditional PI route, preferring instead to focus on their science under the aegis of a well-established PI with the funds and vision to sponsor their innovative work. This model of science permits those exceptional few with creativity, drive, skills, and innovation to work relatively free from the responsibilities in which tenure track/tenured investigators are engaged: grantsmanship, teaching, mentoring, administration, meetings, review panels, and so forth.

Importantly, not having to uproot their families is a distinct advantage for Staff Scientists over the PI route. Most fellows finish their training in their early 30s, at which point, they may have non-scientist spouses (sometimes with more lucrative careers), houses, a community of friends and colleagues, school-age children, and a well-rounded extracurricular life. Postdocs are rejecting the lifestyle that traditional academic life demands. They are no longer willing to uproot their families to start the clock anew as tenure-track investigators, a role which comes with its own attendant risks and rewards. Staff Scientists also have the distinct advantage of having semi-permanent jobs, as long as they succeed in their science and assist the PI in his/her responsibilities. Staff Scientist positions are often associated with large labs with multiple overlapping grants, or institutes (e.g. if they provide a core service required by more than one lab). These jobs can offer impermeability to the vicissitudes of funding cycle dynamics if the PI and institute are firmly established in the academic firmament. Thus, in an era where tenure track positions are difficult to come by and ever more competitive to retain, Staff Scientist positions are popular and highly sought after.

Many Staff Scientists excel in this arena. They have the luxury of time to think deeply about scientific questions, the expertise to design long-ranging experiments, the technical skills to perform the work, the intellectual training to analyze the data, and the literary capacity to write manuscripts. Of course, most Staff Scientists do far more than science. They also mentor postdoctoral fellows and graduate students. They develop new tools and technologies. They edit drafts of manuscripts with students. They serve as de facto group head at lab meetings when the PI is away. Some even assist PIs in their teaching responsibilities. Many Staff Scientists author grants as co-PIs and direct their own small team. Thus, in the pursuit of scientific discovery, the job of a Staff Scientist offers the option to take scientific risks and the possibility of fulfilling rewards.

Yamini Dalal , *Ph.D.*
Head, Chromatin Structure and
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The Author's Corner

Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)

Frequent *BAP1* and BRCA Pathway Alterations in Bladder Cancer

Nickerson, M.L., Dancik, G.M., Im, K.M., Edwards, M.G., Turan, S., Brown, J., Ruiz-Rodriguez, C., Owens, C., Costello, J.C., Guo, G., Tsang, S.X., Li, Y., Zhou, Q., Cai, Z., Moore, L.E., Lucia, M.S., Dean, M., Theodorescu, D. (2014) Concurrent alterations in *TERT*, *KDM6A*, and the BRCA Pathway in Bladder Cancer. **Clin Cancer Res** 20(18): 4935-48. PMID: 25225064.

Mike Nickerson, Ph.D., Staff Scientist in the Cancer and Inflammation Program, recently identified somatic *TET2* mutations in prostate cancer using next generation sequencing (NGS) with Michael Dean, Ph.D., and collaborators from The Johns Hopkins University.[1] A fortuitous phone call about the *TET2* results to Dan Theodorescu, M.D., Ph.D., Director, University of Colorado Comprehensive Cancer Center, resulted in a collaboration to examine genetic alterations in bladder cancer (BCa) using NGS and has produced three studies identifying new BCa genes and a new therapeutic target.[2-4]

Bladder cancer is the sixth most common cancer in the U.S. and the fifth most common cancer worldwide, with an estimated 386,300 new cases and 150,200 deaths in 2008. The most common form of BCa is transitional cell carcinoma, composed of superficial (70%) and invasive (30%) subtypes with distinct clinical features. Previous studies of BCa identified mutations in *FGFR3* and Ras family (*HRAS* and *KRAS*) oncogenes, and in *TP53* and *RB1* tumor suppressors. A study led by Beijing Genome Institute (BGI) researchers that included Dr. Nickerson characterized the exomes of nine invasive BCa tumors and matching blood DNA.[2] Somatic nonsynonymous sequence changes were identified in 328 genes which were sequenced in 88 additional tumor-normal pairs to reveal 54 recurrently altered genes. Five had been previously identified (*TP53*, *RB1*, *HRAS*, *KRAS*, and *FGFR3*) and 49 were novel. Sixteen novel genes exhibited mutations in >5% of cases and eight encoded chromatin remodeling proteins, emphasizing a substantial role for altered epigenetic modifiers in BCa. *KDM6A*, a histone 3 lysine 27 demethylase, was the most frequently altered chromatin remodeling gene in 21% of tumors ($p = 2.22 \times 10^{-37}$). *KDM6A* mutations were significantly associated with tumor grade ($p = 0.008$) and were negatively correlated with *HRAS* and *KRAS* mutations, indicating these altered genes may provide similar survival benefits to BCa cells. A second study led by BGI researchers in collaboration with Dr. Nickerson examined the genomes of 99 BCa tumors and matched normal blood DNA from Chinese patients.[3] Thirty-seven genes were significantly mutated, seven were previously associated with BCa, 15 encoded chromatin remodeling proteins, and 13 were new BCa genes. *KDM6A* was the most frequently altered gene in 30% of tumors and 58% of cases displayed alterations in chromatin remodeling genes. The *stromal antigen 2* (*STAG2*) was the most frequently mutated new BCa gene in 11% of cases ($p = 8 \times 10^{-11}$). *STAG2* was also altered by genomic deletions (5%) and promoter CpG hypermethylation (7/30 tumors, 23%). *STAG2* encodes a component of the cohesion complex involved in sister chromatid cohesion and segregation. Analysis of tumor genome copy number variants showed that somatic *STAG2* mutations were associated with higher aneuploidy ($p = 0.01$) and significantly reduced survival by Kaplan-Meier analysis ($p < 0.001$).

The studies above examined patients from a single ethnicity. A third study led by Dr. Nickerson sought to determine if the results were representative also of non-Asian patients. Exome sequencing of 14 BCa tumors from U.S. patients with validation using

The Author's Corner Con't

Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)

Table 1. The genomic complexity of alterations in bladder tumors.

Tumor Type	Variant Status	Tumor ID	Variant Location	Gene	Variant Type	Reference sequence change
Superficial	Somatic	5	exonic	*ARID1A*	stopgain SNV	NM_139135:c.C2323T:p.Q775X
	Somatic	5	exonic	*BAP1*	missense	NM_004656:c.G170A:p.R57Q
	Somatic	5	exonic	*BAP1*	missense	NM_004656:c.G31A:p.D11N
	Somatic	5	exonic	*CHD1*	missense	NM_001270:c.C5050T:p.P1684S
	Somatic	5	exonic	*HUWE1*	missense	NM_031407:c.G12365A:p.R4122H
	Somatic	5	promoter	*TERT*	SNV	
	Somatic	5	exonic	*TSC1*	frameshift deletion	NM_001162427:c.492_510del:p.164_170del
	Somatic	5	exonic	*USP38*	missense	NM_032557:c.T2161A:p.L721I
Superficial	Somatic	7	exonic	*ARID3A*	missense	NM_005224:c.C1211T:p.A404V
	Homozygous germline	7	exonic	*BRCA2*	stopgain SNV	NM_000059:c.A9976T:p.K3326X
	Somatic	7	exonic	*CHD1*	stopgain SNV	NM_001270:c.G814T:p.E272X
	Somatic	7	exonic	*CHD1*	missense	NM_001270:c.G799A:p.E267K
	Somatic	7	exonic	*CSF1R*	stopgain SNV	NM_005211:c.C2198G:p.S733X
	Somatic	7	exonic	*EP300*	CNV loss	
	Somatic	7	exonic	*FLT4*	missense	NM_182925:c.G3962A:p.R1321Q
	Somatic	7	exonic	*HRAS*	missense	NM_001130442:c.G34A:p.G12S
	Somatic	7	exonic	*HTT*	missense	NM_002111:c.A1874G:p.Q625R
	Somatic	7	exonic	*KDM6A*	stopgain SNV	NM_021140:c.C3634T:p.Q1212X
	Somatic	7	exonic	*MDM1*	stopgain SNV	NM_017440:c.C1927T:p.R643X
	Somatic	7	exonic	*SETDB2*	missense	NM_001160308:c.C471G:p.I157M
	Somatic	7	promoter	*TERT*	SNV	
Superficial	Germline	9	exonic	*AHNAK*	nonframeshift insertion	NM_024060:c.8_9insAGG:p.K3delinsKE
	Somatic	9	splicing	*ATM*	IVS-1, completely conserved across species	
	Somatic	9	exonic	*ATM*	missense	NM_000051:c.A7343G:p.D2448G
	Somatic	9	exonic	*BAP1*	missense	NM_004656:c.C507G:p.H169Q
	Somatic	9	exonic	*DNMT1*	missense	NM_001379:c.G1016C:p.R339P
	Somatic	9	exonic	*ERBB3*	CNV loss	
	Germline	9	exonic	*FGFBP1*	frameshift deletion	NM_005130:c.6delG:p.K2fs
	Somatic	9	exonic	*KDM6A*	missense	NM_021140:c.T3403G:p.Y1135D
	Somatic	9	exonic	*KDM6A*	nonframeshift insertion	NM_021140:c.3403_3404insTAG:p.Y1135delins
	Somatic	9	promoter	*TERT*	CNV amplification	
Invasive	Somatic	1	exonic	*BAP1*	missense	NM_004656:c.A506G:p.H169R
	Somatic	1	exonic	*ERBB3*	nonframeshift deletion	NM_001982:c.3358_3369del:p.1120_1123del
	Somatic	1	exonic	*FGFR3*	missense	NM_000142:c.C746G:p.S249C
	Somatic	1	exonic	*GCN1L1*	missense	NM_006836:c.C7770G:p.I2590M
	Somatic	1	exonic	*GCN1L1*	missense	NM_006836:c.C7722G:p.I2574M
	Somatic	1	exonic	*SYNE1*	missense	NM_033071:c.G12738T:p.Q4246H
	Somatic	1	promoter	*TERT*	SNV	
Invasive	Somatic	3	exonic	*ANK3*	missense	NM_020987:c.C5309T:p.T1770M
	Somatic	3	exonic	*BRCA1*	missense	NM_007294:c.C1090G:p.P364A
	Somatic	3	exonic	*CABIN1*	missense	NM_001199281:c.6517_6519del:p.2173_2173del
	Somatic	3	exonic	*CHD1*	missense	NM_001270:c.C5050T:p.P1684S
	Somatic	3	exonic	*ITGA10*	missense	NM_003637:c.C2851T:p.R951C
	Somatic	3	exonic	*NCOA1*	missense	NM_003743:c.G3846A:p.M1282I
	Germline	3	exonic	*RB1*	frameshift insertion	NM_000321:c.2537_2538insA:p.Q846fs
	Somatic	3	exonic	*RSF1*	missense	NM_016578:c.G3488A:p.R1163Q
	Somatic	3	promoter	*TERT*	SNV	
	Somatic	3	exonic	*TP53*	missense	NM_001126114:c.G853A:p.E285K
Invasive	Germline	14	exonic	*ALDH1B1*	frameshift deletion	NM_000692:c.1161delC:p.G387fs
	Germline	14	exonic	*ALDH1B1*	frameshift deletion	NM_000692:c.578delG:p.G193fs
	Somatic	14	exonic	*ATM*	CNV loss	
	Somatic	14	exonic	*KDM6A*	frameshift deletion	NM_021140:c.219_225del:p.73_75del
	Somatic	14	exonic	*LRP1B*	missense	NM_018557:c.G1592A:p.R531H
	Somatic	14	exonic	*NCOR1*	missense	NM_001190440:c.C2491G:p.P831A
	Somatic	14	exonic	*SYNE2*	stopgain SNV	NM_015180:c.G1140A:p.W380X
	Somatic	14	promoter	*TERT*	SNV	

Selected germline and somatic alterations are shown. Red, putative/likely tumor suppressor; green, putative/likely oncogene. Boxes, homozygous mutants due to >1 sequence changes, allele loss or amplification, or an X chromosome alteration in males. SNV, single nucleotide variant; CNV, copy number variation.

The Author's Corner Con't

Section Editor: Cristina Bergamaschi, Ph.D. (*SS*)

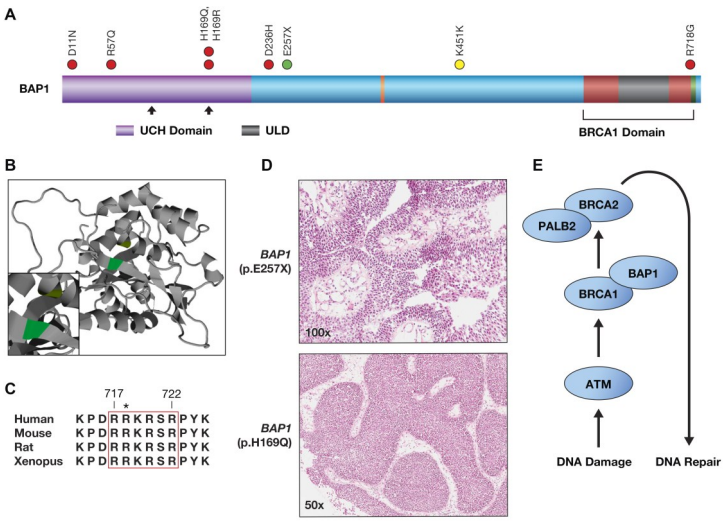

***Figure 1. BAP1* and BRCA pathway alterations in bladder cancer. A**, Somatic *BAP1* mutations in 8/54 (15%) bladder tumors. **B**, A ribbon diagram showing the recurrently-altered proton donor residue in the enzyme active site, p.H169 (green). **C**, Altered residue, p.R718 (*), in the nuclear localization signal (box) and BRCA1 binding domain, is highly conserved. **D**, Papillary features in H&E stained tumor sections from *BAP1*-mutant tumors. **E**, BRCA pathway proteins encoded by altered BCa genes.

Mike Nickerson is a Staff Scientist in the Cancer and Inflammation Program, NCI, conducting research examining the genetic basis of urologic cancer. He initiates new projects, supervises ongoing projects, and writes manuscripts for publication. He has trained graduate and undergraduate students to accurately and comprehensively conduct genetic analyses of patient samples, including as a Thesis Advisor for four students in the Biomedical Master's degree program, Hood College; and as a mentor for Cancer Summer Interns and Werner Kirsten Student Interns. He has established collaborations with researchers in the Division of Cancer Epidemiology and Genetics, NCI; Urologic Oncology Branch, NCI; Advanced Technology Research Facility; Johns Hopkins University; University of Colorado; Louisiana State University; and the National Heart, Lung, and Blood Institute.

matching normal tissue DNA[4] revealed 228 variants in recurrently-altered genes, 112 were somatic and 116 were germline. Variants in known or likely cancer and disease-associated genes demonstrate the genetic complexity of BCa (Table 1). In 14 tumors, 43 germline alleles were novel and 11 were deleterious nonsense, frameshift, and splice junction alterations in known and putative cancer genes. This suggests rare germline variants make a significant contribution to 'sporadic' BCa. The *telomerase* (*TERT*) promoterwas altered by complex combinations of somatic and germline nucleotide substitutions in 69% and 56% of tumors, respectively, implicating *TERT* as the most frequently mutated BCa gene. *TERT* somatic and germline variants created new and altered existing transcription factor binding sites, and altered CpG methylation sites, potentially disregulating gene expression. Somatic alterations were observed in 67 genes, including four novel BCa genes encoding chromatin remodeling proteins, *GCN1L1*, *CHD1*, *CHD1L*, and the *BRCA1 associated protein-1* (*BAP1*). Fifteen % of 54 tumors displayed somatic *BAP1* alterations that introduced a premature stop codon and altered the proton donor residue in the ubiquitin hydrolase active site and the nuclear localization signal (Figure 1). *BAP1* mutations co-occurred with *KDM6A* mutations (p = 0.017) and contributed to a high frequency of tumors with BRCA DNA repair pathway alterations. Germline and somatic alterations targeting the BRCA pathway in 10/14 (71%) tumors altered *BRCA1*, *BRCA2*, *ATM*, *PALB2*, and *BAP1*, indicating a significant role for altered DNA repair in BCa. *BAP1* mutations were associated with papillary histologic features in some BCa tumors and occurred more frequently in patient tumors from U.S. as compared to China (15% vs. 1%t; p = 0.003). However, three other frequently mutated genes, *ARID1A*, *KDM6A*, and *STAG2*, were similarly altered in the two populations, indicating *BAP1* mutations in U.S. patients may be due to differences in genetics, lifestyle, or exposure.

These studies identify frequently altered cancer genes that can be mechanistically associated with canonical features of BCa, such as aneuploidy (*STAG2*), papillary tumor histology (*BAP1*), and lethal disease (*STAG2*). Frequent alteration of two BCa genes on the X chromosome, *KDM6A* and *STAG2*,

are single copy in males and likely contribute to the gender bias observed in bladder and other cancers. These studies reveal previously unappreciated interactions between altered cancer genes, such as frequent alterations of genes encoding BRCA pathway proteins, which suggest a therapeutic opportunity. *BRCA1*- and *BRCA2*-mutant breast, ovarian, and prostate cancer are responsive to poly(ADP-ribose) polymerase (PARP) inhibitors such as FDA-approved olaparib, indicating PARP inhibitors may be effective in BRCA pathway-mutant BCa.[5,6]

Dr. Nickerson would like to acknowledge the significant contributions to these studies from many collaborators, students, and core laboratories at the NCI.

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References:

1. **Nickerson, M.L.**, Im, K.M., Misner, K.J., Tan, W., Lou, H., Gold, B., Wells, D.W., Bravo, H.C., Fredrikson, K., Harkins T., Milos, P., Zbar, B., Linehan, W.M., Yeager, M., Andresson, T., Dean, M., and Bova, G.S. (2013). Somatic alterations contributing to metastasis of a castration-resistant prostate cancer. *Human Mutation* 34(9): 1231-41.

2. Gui, Y., Guo, G., Huang, Y., Hu, X., Tang, A., Gao, S., Wu, R., Chen, C., Li, X., Zhou, L., He, M., Li, Z., Sun, X., Jia, W., Chen, J., Yang, S., Zhou, F., Zhao, X., Wan, S., Ye, R., Liang, C., Liu, Z., Huang, P., Liu, C., Jiang, H., Wang, Y., Zheng, H., Sun, L., Liu, X., Jiang, Z., Feng, D., Chen, J., Wu, S., Zou, J., Zhang, Z., Yang, R., Zhao, J., Xu, C., Yin, W., Guan, Z., Ye, J., Zhang, H., Li, J., Kristiansen, K., **Nickerson, M.L.**, Theodorescu, .D, Li, Y., Zhang, X., Li, S., Wang, J., Yang, H., Wang, J., and Cai, Z. (2011). Frequent mutations of chromatin remodeling genes in transitional cell carcinoma of the bladder. *Nature Genetics* 43(9): 875-78.

3. Guo, G., Sun, X., Chen, C., Wu, S., Huang, P., Li, Z., Huang, Y., Jia, W., Zhou, Q., Tang, A., Yang, Z., Li, X., Song, P., Zhao, X., Ye, R., Zhang, S., Lin, Z., Qi, M., Wan, S., Xie, L., Fan, F., **Nickerson, M.L.**, Dean, M., Zou, X., Hu, X., Mei, H., Gao, S., Liang, C., Gao, Z., Lu, J., Yu, Y., Liu, C., Li, L., Jiang, Z., Yang, J., Li, C., Zhao, X., Chen, J., Zhang, F., Lai, Y., Lin, Z., Zhou, F., Theodorescu, D., Li, Y., Zhang, X., Wang, J., Yang, H., Gui, Y., Wang, J., and Cai, Z. (2013). Whole-genome and whole-exome sequencing of bladder cancer identifies frequent alterations in genes involved in sister chromatid cohesion and segregation. *Nature Genetics* 45(12): 1459-63.

4. **Nickerson, M.L.**, Dancik, G.M., Im, K.M., Edwards, M.G., Turan, S., Brown, J., Ruiz-Rodriguez, C., Owens, C., Costello, J.C., Guo, G., Tsang, S.X., Li, Y., Zhou, Q., Cai, Z., Moore, L.E., Lucia, M.S., Dean, M., Theodorescu, D. (2014) Concurrent alterations in *TERT*, *KDM6A*, and the BRCA Pathway in Bladder Cancer. *Clinical Cancer Research* 20(18): 4935-48.

5. Audeh, M.W., Carmichael, J., Penson, R.T., Friedlander, M., Powell, B., Bell-McGuinn, K.M., Scott, C., Weitzel, J.N., Oaknin, A., Loman, N., Lu, K., Schmutzler, R.K., Matulonis, U., Wickens, M., Tutt, A. (2010). Oral poly(ADP-ribose) polymerase inhibitor olaparib in patients with *BRCA1* or *BRCA2* mutations and recurrent ovarian cancer: a proof-of-concept trial. *The Lancet* 376: 245-51.

6. http://uk.reuters.com/article/2014/11/04/us-astrazeneca-cancer-idUKKBN0IO1QE20141104.

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