The Council of Biomedical Graduate Students

2019 Graduate Research Forum

COLLEGE OF MEDICINE AND LIFE SCIENCES
THE UNIVERSITY OF TOLEDO

BIOMEDICAL SCIENCES GRADUATE PROGRAM
THE UNIVERSITY OF TOLEDO

Sponsored by:

GRADUATE STUDENT ASSOCIATION

Graduate Research Forum

March 20th-21st, 2019

Hosted by CBGS

Keynote Speaker

Janet Iwasa, Ph.D.

presents

"Animating Biology"

Assistant Professor of Biochemistry,
The University of Utah
Molecular and cellular visualizations
2014 TED fellow, and 2017 TED senior fellow

Wednesday March 20th

Location: Health Science Campus Mulford Library Café

- Poster Presentations: 10am-12pm
- Lunch: 12pm-1pm
- Oral Presentations: 1pm-4pm

Thursday March 21st

- Final Presentations: 9:30am-11:45am
- Lunch with Keynote Speaker: 12:00pm-1pm, Mulford Café
- **Keynote Speech: 3:00pm Collier 1000A/B**

If you wish to have lunch with Dr. Iwasa, please RSVP to CouncilGraduateStudents@utoledo.edu by March 15th

Funded by

GRADUATE STUDENT ASSOCIATION

2019 Graduate Research Forum

March 20th-21st
Mulford Library Café (028A)
Health Science Campus

Wednesday, March 20th

Time	*Event*	*Location*
10:00 AM – 12:00 PM	Preliminary Poster Session	Mulford Café
12:00 PM – 1:00 PM	Lunch	Mulford Café
1:00 PM – 4:00 PM	Preliminary Oral Sessions	Mulford Café

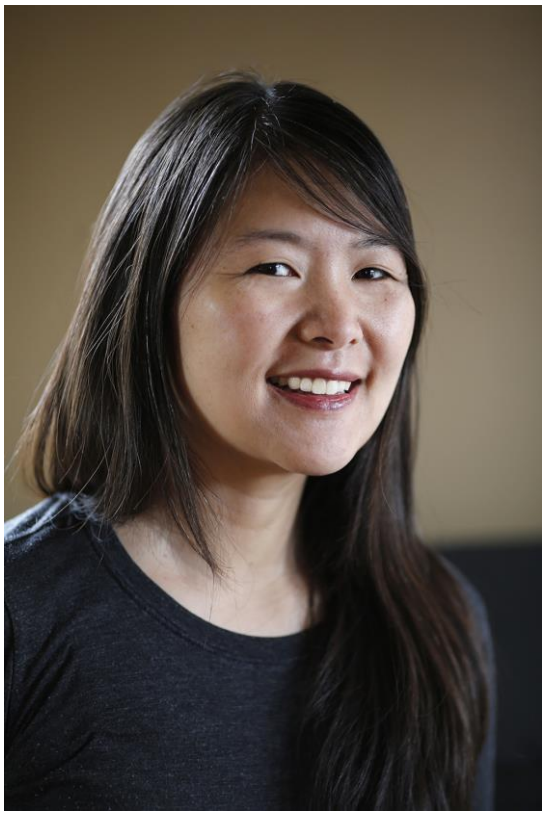
Thursday, March 21st

Time	*Event*	*Location*
9:45 AM – 10:45 AM	Final Poster Session *(closed to public)*	Mulford Café
11:00 AM – 12:00 PM	Final Oral Session *(closed to public)*	Mulford Café
12:00 PM – 1:00 PM	Lunch with Dr. Janet Iwasa *(RSVP'd participants only)*	Mulford Café
3:00 PM – 3:30 PM	Keynote Reception	COB Lobby
3:30 PM – 4:30 PM	Keynote Lecture	COB 1000A/B

Keynote Lecture

"Animating Biology"

2019 Keynote Speaker



Janet Iwasa, Ph.D.
Assistant Professor of Biochemistry
The University of Utah
Molecular and Cellular Visualizations
2014 TED fellow and 2017 TED senior fellow

Dr. Iwasa's lab focuses on creating information-rich and visually compelling animations that capture current hypotheses on diverse molecular and cellular processes.

The Council of Biomedical Graduate Students

The University of Toledo Council of Biomedical Graduate Students consists of officers and representatives from the College of Medicine and Life Sciences and the College of Pharmacy and Pharmaceutical Sciences at the University of Toledo. This includes the Biomedical Science Graduate Program and related graduate programs in Pharmacy, Medicinal & Biological Chemistry, and from the Center of Excellence in Biomarker Research & Individualized Medicine (BRIM) at the Health Science Campus.

The overall purpose of the Council is to facilitate discussion amongst graduate students pertaining to any issue that may affect graduate life; to represent graduate student interests before the UT faculty, GSA (our main campus counterparts), and administration; and to organize events and activities beneficial to graduate student life.

We meet regularly, at least once per month, to discuss any current issues that need to be addressed and to plan and organize upcoming events. **The meetings are open to all graduate students** to encourage discussion of ideas and concerns pertaining to graduate student life. However, only elected members of the Council may vote during the meetings.

Annual events organized by the CBGS include:

- **Graduate Student Picnic** - A summer social event for new and current students
- **Career Forum** - Held in autumn to help guide students for career decisions
- **Graduate Research Forum** - Held during the spring semester to allow students to showcase their research and get helpful advice from faculty and fellow students

Visit us at http://www.utoledo.edu/med/grad/biomedical/cbgs/

The CBGS would like to thank all the volunteers, judges, and the UT Graduate Student Association (GSA). This year's forum was FULLY sponsored by GSA. The forum could not happen without all of you. THANK YOU!

GRADUATE STUDENT ASSOCIATION

Poster Presentations

GROUP 1	Briana Zellner	P1
	Sangita Sridharan	P2
	Shungang Zhang	P3
	Khaled Alganem	P4
	Joshua Letson	P5
	Mariah Pasternak	P6
GROUP 2	Cory Howard	P7
	Ahmed Al-Khudhair	P8
	Swapnaa Balaji	P9
	Saroj Chakraborty	P10
	Daniyah Almarghalani	P11
	Irum Syed	P12
GROUP 3	Kelsey Murphy	P13
	Yashna Walia	P14
	Rachel Golonka	P15
	Chrysan Mohammed	P16
	Sara Moore	P17
	Sayani Bhattacharjee	P18
GROUP 4	Smrithi Sugumaran Menon	P19
	Fatimah Khalaf	P20
	Kathryn Becker	P21
	Cara Peter	P22
	Amsha Alsegiani	P23
	Jonnelle Edwards	P24
GROUP 5	Jin Chen	P25
	Scott Miruzzi	P26
	Hallie Dolin	P27
	Amit Sopan Chougule	P28
	Augustus Tilley	P29
	Justin Creeden	P30
	Shermel Sherman	P31

Poster Contest Procedure

- See presentation guidelines on page 8.
- Preliminary Session- Each group will be judged by three faculty members and one finalist will be selected from each group.
- Final Session- Finalists will present to:
  - Dr. Janet Iwasa, 2019 GRF Keynote Speaker
  - Dr. Kandace Williams, Associate Dean for College of Medicine & Life Sciences
  - Dr. Frederick E. Williams, Chair Dept. of Pharmacology and Experimental Therapeutics
- The top three presenters will be awarded:
  - $300 for first place
  - $200 for second place
  - $100 for third place

Oral Presentations

GROUP A	Sandun Kalpana	O1
1:00-2:00 PM	Apurva Lad	O2
	Omar N. Issa	O3
	Sarah Galla	O4
GROUP B	Deepti Gurung	O5
2:00-3:00 PM	Robin Su	O6
	Neha Nandedkar-Kulkarni	O7
	Federico Resendiz	O8
GROUP C	Rajan Paudel	O9
3:00-4:00 PM	Noor Hussein	O10
	Gayatri Subramanian	O11
	Usman Ashraf	O12

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Guidelines for Poster and Oral Presentations

POSTER PRESENTATIONS

1. Each poster presentation will be given a maximum of 15 minutes, including time for questions. Presenters are advised to limit their explanation of posters to 10 minutes (max 12 minutes) to allow time for questions.
2. Poster boards (size - 3 feet high and 4 feet wide) will be provided. Whether or not you have a professionally printed poster will not affect your scores in any way. However, please make sure all text, figures and diagrams are clear and legible. If you wish to show data in the form of videos, please inform CBGS members one day before the forum via email. The students are responsible for bringing their own devices (laptop, ipad, etc.) to show their videos (and making sure it is adequately charged). Although the council will try to help the student as far as possible, the council is not responsible for the electronic devices (i.e. losing battery etc.).
3. Each presenter will be given a number for their poster. All presenters are requested to put up their posters on their respective poster board (presenters will be informed of their poster numbers prior to the forum).
4. Judging Criteria: The judging will be based on knowledge of the subject, explanation of background and significance, organization of poster, clarity of presentation, conclusions supported by data, ability to finish presentation in allotted time, ability to answer questions and overall presentation.

ORAL PRESENTATIONS

1. Each oral talk will be given a maximum of 15 minutes including time for questions. Presenters are advised to limit their presentations to 10 minutes (max 12 minutes) to allow time for questions.
2. There is no restriction for the number of slides per presentation. Please make sure all text, figures and diagrams on the slides are clear and legible.
3. All presenters are requested to hand over their presentations (in a USB drive) to their respective session moderators at least 15 minutes prior to the start of the oral presentation sessions. (Presenters will be informed of their sessions prior to the forum.)
4. Judging Criteria**:** Judging will be based on knowledge of the subject, organization and clarity of the talk, conclusions supported by data, ability to finish the talk in allotted time, ability to answer questions, and overall presentation.

Poster Presentation Abstracts

P1: **A *Francisella tularensis* LD-Carboxypeptidase is Involved in Cell Wall Repair and Virulence**

B. Zellner, D. Mengin-Lecreulx, and J.F. Huntley

Francisella tularensis (*Ft*), the causative agent of tularemia, is one of the most dangerous bacterial pathogens known. Due to its low infectious dose, ease of aerosolization, and high morbidity and mortality, it has been designated as a Tier 1 Select Agent. Characterization of *Ft* envelope proteins are important to help understand the molecular mechanisms by which *F*t and other intracellular pathogens cause disease. In previous studies, we demonstrated that the *Ft* disulfide bond formation protein ortholog, DsbA, is required for virulence and, more importantly, identified >50 DsbA substrates, half of which are annotated as hypothetical proteins. In the current study, we selected one of these unstudied DsbA substrates, FTL1678, for detailed analysis. Using bioinformatics, FTL1678 was found to contain a putative LD-carboxypeptidase domain, indicating a potential role in peptidoglycan recycling. An FTL1678 mutant was completely attenuated in a mouse pulmonary infection model, with decreased lung colonization and inability to disseminate to livers or spleens. Mutant attenuation was confirmed through complementation, which fully-restored virulence to wild-type (WT) levels. Importantly, immunization with this mutant provided significant protection against pulmonary challenge with fully-virulent *Ft* strain SchuS4 (BSL3). Membrane integrity testing revealed differences in cell wall permeability between WT and ΔFTL1678. Additionally, electron microscopy analysis of ΔFTL1678 showed increased cell wall thickness. LD-carboxypeptidase activity of FTL1678 was confirmed through enzymatic assays utilizing various peptidoglycan substrates. Current studies are examining immune responses (humoral and cell-mediated) to the WT and mutant and analyzing peptidoglycan composition of WT and mutant.

P2: **Targeted therapy against protein translation initiation machinery in breast cancer stem-cells**

Sangita Sridharan, and Dayanidhi Raman

Breast cancer represents one of the major fatalities worldwide, claiming a staggering 15% of all the cancer related deaths in women. It is often not the primary tumor itself, but recurrence and metastasis leading to high mortality rates due to breast cancer. Recurrence can often be attributed to the presence of residual cancer stem-like cells (CSCs) that persist even after the chemotherapeutic regimen. Also termed as tumor initiating cells, these cancer stem-like cells contribute significantly to the tumor landscape owing to their unparalleled ability to recapitulate the heterogeneous bulk tumor and relative quiescence, thereby endowing them with the ability of being refractory to chemo/radio-therapeutics. This residual population forms a major reason why monotherapy fails invariably in the clinics and patients relapse. Therefore, combinatorial approaches co-targeting the bulk tumor and CSCs will be necessary. Towards that effect, we have been successful in isolating and characterizing distinct stem cell populations that are positive for CSC markers - aldehyde dehydrogenase (ALDH+) and CD44+/CD24- population Towards that effect, we have been successful in isolating and characterizing distinct cancer stem-cell populations that are positive for CSC markers - aldehyde dehydrogenase (ALDH+) and CD44+/CD24- population. Eliminating both the types of CSCs is imperative to prevent relapse. Therefore, common mechanisms that could be exploited to attack both the populations need to be explored. One such mechanism that we are exploring is protein translation initiation machinery. We have also generated a paclitaxel-resistant cell line to challenge with novel chemical inhibitors. This will be employed in a chemical screen to identify novel drugs/inhibitors that could co-target bulk tumor cells, naïve and the drug-resistant CSCs. The promising compounds would be then translationally tested in pre-clinical studies.

P3: **Targeted Disruption of CD40 in Human Proximal Tubular Epithelial Cells Significantly Reduces Pro-inflammatory and Pro-fibrotic Signaling**

Shungang Zhang, Terry D. Hinds, Fatimah K. Khalaf, Apurva Lad, Andrew Kleinhenz, Deepak Malhotra, David J. Kennedy, and Steven T. Haller.

Background: We have demonstrated that the TNF-α superfamily member CD40 significantly contributes to the development of renal injury clinically in patients with chronic kidney disease and using our novel Cd40 mutant model in which Cd40 function is abolished. To test the hypothesis that activation of CD40 in the proximal tubule epithelium induces a significant pro-inflammatory and pro-fibrotic response, we treated human proximal tubular epithelial cells (HK2 cells) with soluble CD40 ligand (sCD40L). In addition, a novel CD40-trageted peptide to inhibit CD40 signaling and a HK2 CD40-knockout (HK2/CD40KO) cell line were used to detect the responses when CD40 signaling is disrupted.

Methods: HK2 cells were treated with sCD40L (100ng/ml) for 24h. Cytokine secretion from HK2 cells was detected using an antibody-conjugated cytokine secretion assay. A CD40-targeted peptide (1ug/ml, 10ug/ml and 100ug/ml) was incubated in the presence and absence of sCD40L prior to treatment. CRISPR/Cas9 was used to create the HK2/CD40KO cell line. Parent HK2 cells and HK2/CD40KO cells were treated with sCD40L (100 ng/ml) or TNF-α (10 ng/ml) for 24h.

Results: In HK2 cells, pro-inflammatory and pro-fibrotic gene expression was significantly upregulated following sCD40L treatment compared to control and TNF-α treatment. This response was significantly attenuated in the HK2/CD40KO cells ($p<0.05$). Co-treatment with the CD40-target peptide significantly reduced monocyte chemotactic protein-1 expression by three-fold compared to sCD40L treatment alone ($p<0.01$).

Conclusion: Activation of CD40 in the proximal tubule epithelium induces a significant pro-inflammatory and pro-fibrotic response, and represents an attractive therapeutic target for the treatment of renal disease.

P4: **Kaleidoscope: A New Bioinformatics Pipeline Application for in Silico Hypothesis Testing of Gene Expression Changes in Severe Mental Illness**

Khaled Alganem, Scott Miruzzi, Jarek Meller, and Robert McCullumsmith.

Background: By taking advantage of robust bioinformatics tools and rich biological databases, interesting information can be obtained that could be very valuable to the field of biological psychiatry. Integrating multiple databases and tools and exploring them through a cohesive bioinformatics pipeline can lead to exciting observations which could supplement existing hypotheses, spawn new ones and possibly direct future studies. There are a large number of bioinformatics tools and databases that are relevant to psychiatric disorders and studies that are relatively untapped. We addressed this opportunity by developing a platform that provides easy access to these tools and datasets with a user-friendly interface that could be explored by researchers to test hypotheses in silico.

Methods: An interactive web application (called Kaleidoscope) was developed to serve as a platform for exploratory data analysis and in silico analysis for relevant neuroscience fields. This web application was developed using the R Shiny coding platform. The application takes advantage of online application programming interfaces (APIs) and publicly available datasets. The bioinformatics tools connected to our software include Brain RNAseq, Brain Cloud, STRING, iLINCS and Allen Brain Atlas. Fold-change values for mRNAs and proteins from microarray, RNAseq, and mass spectrometry datasets are harmonized for side by side comparison with visual interpretation using scalable heat maps. Our initial launch includes access to more than 15 schizophrenia versus control databases covering diverse brain regions, cell types, and tissue sources.

Results: We have successfully deployed this software for two manuscripts. We performed confirmation analyses for a cell-subtype specific study of glycolytic genes in schizophrenia. We grew a protein interaction network around the target proteins, confirmed changes in mRNA expression for some of the hits, and generated drug candidates using transcriptional profiling in iLINCs. In the second, unrelated study, we used Kaleidoscope to explore changes in protein kinase mRNAs, as well as their up and downstream targets, across multiple databases, confirming our reported changes in protein kinase activity.

Conclusion: The platform can be utilized in many forms and offers great scalability with the option of uploading user defined datasets for comparison alongside the pre-defined datasets. While out program is still in development, it is available for download on github (https://kalganem.shinyapps.io/SCZ_Databases_F/). This tool will facilitate introduction of bioinformatics approaches to biological psychiatrists, promoting educated use of bioinformatics pipelines in our field.

P5: **The Role of Nitric Oxide in Modulating the TGFβ / LTBP1 Complex**

Joshua Letson, and Saori Furuta

TGFβ is a ubiquitous signaling molecule that is involved in many biological activities including cell proliferation, differentiation and induction of apoptosis. The activity of TGFβ is primarily regulated post-translationally. Nascent TGFβ is usually secreted in an inactive confirmation and will form a complex with latent transforming growth factor β binding protein-1 (LTBP1) called the large latent complex. This complex is responsible for keeping the TGFβ protein bound in its inactive conformation. While some pathways have been discovered as to how this inactive, complex-bound protein can become activated, we hypothesize that a decrease in the level of nitric oxide (NO) can lead to release and eventual activation of the TGFβ pathway. In breast cancer, we hypothesize that these low levels of NO seen throughout breast cancer cell lines are responsible for the release of TGFβ. This heightened activation of TGFβ can then lead to progression of the disease. In breast cancer, TGFβ signaling is upregulated, resulting in an increase of ECM proteins. This causes stiffer ECM which may promote tumorigenesis. By restoring the level of NO back to physiological conditions, we hypothesize that TGFβ activity will be greatly reduced. NO is able to interact with various proteins via a post-translational modification known as s-nitrosylation. We believe that both TGFβ and LTBP1 can both undergo this modification resulting in their inactive bound state. We have shown that both proteins indeed undergo a high level of s-nitrosylation in a breast cancer progression series cell line.

P6: **Development of small molecules that induces a novel caspase-independent cell death**

Mariah A. Pasternak, Noor Hussein, Diwakar B. Tukaramrao, Paul W. Erhardt, and Amit K. Tiwari

Since most cancer therapeutics currently exert their effects using apoptotic pathways to which cancer cells can become resistant, it is important to develop new drug molecules that work through non-apoptotic pathways. A novel, non-apoptotic cell death mechanism called “methuophagy,” which combines features of both autophagy and methuosis, is being examined using structure-activity relationship (SAR) studies of synthesized compounds. Methuosis is a non-apoptotic cell death that includes macropinocytosis and the fusing of smaller vesicles into larger vesicles that is seen along with cell death. BAPT compounds that induce methuophagy have been synthesized, and minor changes on a primary scaffold are being used to determine the SAR for the series. The series retains the 4-pyridinyl moiety that induced methuosis with the compound MOMIPP. Screening experiments with HCT-116 colorectal cancer cells (CRC), BT-20 triple-negative breast cancer cells (TNBC), and U-251 glioblastoma cancer cells (GBM) have shown that compounds having a rigid, completely aromatic structure with three aromatic centers induce methuophagy more potently than compounds with a “hydrazone-like” moiety or a non-aromatic linker. The cell death caused by methuophagy can be seen as early as 6 hours, with small vacuoles that coalesce together to form larger vacuoles. From these screening experiments, it has been concluded that the completely aromatic compounds are the most promising candidates for methuophagy drug development and will be used in experiments to determine protein targets and in vivo to further determine efficacy.

P7: **Understanding the Role of LASP1 in Triple Negative Breast Cancer**

Cory M. Howard, Nicole Bearss, Boopathi Subramaniyan, Augustus Tilley, Sangita Sridharan, Nancy Villa, Christopher S. Fraser, and Dayanidhi Raman

Triple negative breast cancer (TNBC) remains clinically challenging as effective targeted therapies are lacking. In addition, patient mortality mainly results from the metastasized lesions. CXCR4 has been identified to be one of the major chemokine receptors involved in breast cancer metastasis. Previously, our lab had identified LIM and SH3 Protein 1 (LASP1) to be a key mediator in CXCR4-driven invasion. Our mechanistic investigation of such invasion led to the identification of a novel protein-protein interaction involving LASP1. We hypothesize that activation of the CXCR4-LASP1-axis promotes this novel interaction. As a result, breast cancer progression and metastasis is facilitated. Overall, our work identifies innovative functions for both CXCR4 and LASP1. Thus, our axis of study represents a potential target for future TNBC therapies.

P8: **Type 1 Diabetes association with the Program Death Ligand1 gene (PDL1), and a therapeutic approach to improve islets transplantation**.

Al-Khudhair Ahmed, Pervaiz Dar, Shahnawaz Imam, and Juan C. Jaume.

The Programmed cell death-1 (PD-1) and its ligand (PD-L1) pathway believed to play an important role in maintaining immune tolerance in the pancreatic islets. Genetic studies revealed SNPs association between PD-1 and PD-L1 and many autoimmune diseases including T1D. A newly rising evidence comes from many cases of T1D as a side effect after administering PD1 pathway blocker as a treatment for cancer. Evidences all together raised our inquisitiveness to investigate the possibility of intensifying self-tolerance by overexpressing the PD-L1 molecule in the pancreatic islets, accordingly may allow enhanced posttransplantation survival of islet allografts in the T1D mice model. We achieved an ex-vivo PD-L1 gene delivery into the islets by utilizing a Lentivirus vector (Lt-PDL1), Islets are effectively transduced and confirmed for their overexpressing of PD-L1 using immunofluorescence confocal microscopy and by western blot. Further downstream experiments are being conducted to confirm the viability and possible altering glucose stimulating insulin secretion of these islets. The in vivo transduced islets graft survival is going to be tested in a T1D humanized transgenic mice, this humanized mice model is characterized by antigen-specific activation of the immune system targeting pancreatic beta cells, which can mimic the recurrent autoimmune damage after isograft and allograft transplantation.

P9: **Analysis of underivatized amino acids in the serum by liquid chromatography tandem mass spectrometry- a metabolomic study**

Swapnaa Balaji and Marcia F. McInerney

The role of insulitis in the etiopathogenesis of insulin- dependent diabetes mellitus (IDDM) is well established. However, our knowledge about the exact mechanisms involved in the triggering of the T-cell mediated inflammatory response is still scarce. It is generally acknowledged that both genetic and environmental perturbations are involved in the progression of the disease state. Having known that the metabolomic phenotype is highly sensitive to minor variations in both the genetic and environmental factors, it is only pragmatic to exploit it to gain insights into the premature stages of the disease, the knowledge of which could further be applied to the development of biomarkers that could pave way for better preventive strategies.

This study intends at defining amino acids as potential biomarkers which could help develop better interventional strategies in the diagnosis and prevention of the disease state at an early stage. Of particular interest we would like to show metabolic phenotypic variations in our transgenic mice that could provide us evidence that IR overexpressing T cell infiltration in the pancreas can actually have an effect on the amino acid metabolism.

As LC-MS is often celebrated as the most favored analytical technique for quantitation of compounds owing to its high definition sensitivity and selectivity, it is obvious that this is what is to be used for the analysis of amino acids, each of which have an average molecular weight of about 110 Da.

P10: **Salt-Responsive Metabolite, β-Hydroxybutyrate, Attenuates Hypertension**

Chakraborty S, Galla S, Cheng X, Yeo JY, Mell B, Singh V, Yeoh B, Saha P, Mathew AV, Vijay-Kumar M, and Joe B

Dietary salt reduction and exercise are lifestyle modifications for salt-sensitive hypertensives. While exercise has prominent metabolic effects, salt has an adverse effect on metabolic syndrome, of which hypertension is a hallmark. We hypothesized that dietary salt impacts metabolism in a salt-sensitive model of hypertension. An untargeted metabolomics approach demonstrates lower circulating levels of the ketone body, beta-hydroxybutyrate (βOHB), in high salt-fed hypertensive rats. Despite the high salt intake, specific rescue of βOHB levels by nutritional supplementation of its precursor, 1,3-butanediol, attenuates hypertension and protects kidney function. This beneficial effect of bOHB was likely independent of gut-microbiotal and Th17-mediated effects of salt and instead facilitated by βOHB inhibiting the renal Nlrp3 inflammasome. The juxtaposed effects of dietary salt and exercise on salt-sensitive hypertension, which decrease and increase βOHB respectively, indicate that nutritional supplementation of a precursor of βOHB provides a similar benefit to salt-sensitive hypertension as exercise.

P11: **The role of Cofilin in Intracerebral Hemorrhage-induced Neuroinflammation**.

Daniyah Almargalani, Mohammad Amira, Ghaith Bahader, Jose Sola, Devin Ta, Akhila Kondaka, and Zahoor A. Shah.

Intracerebral hemorrhage (ICH), a devastating type of stroke accounts for about 10-15% of all stroke cases and is associated with high mortality. The understanding about the pathological changes and the repair mechanisms after ICH are not clearly understood. Our previous study demonstrated a major role of cytoskeletal protein, cofilin in ICH-induced brain injury. We reported that knockdown of cofilin in a mouse model of collagenase induced-ICH improved neurobehavioral deficits and decreased hemorrhagic volume as well as blood-brain barrier disruption (BBB) and microglia activation. In this study, we aimed to evaluate the cofilin signaling longitudinally up to 28 days following ICH. We, therefore, subjected different cohorts of mice to intra-striatal collagenase injection-induced ICH and mice were sacrificed at different time-points of 1, 3, 7, 14, 21, and 28 days. Using Western blotting, we observed a significant upregulation of cofilin protein in the ipsilateral striatum on day 3 and then a decreasing trend was observed from day 7 to day 28. Mice suffered from severe neurobehavioral deficits immediately after ICH which lasted for 7 days and then a gradual improvement was observed in motor deficits which lasted up to 28 day. Using immunohistochemistry analysis, activated microglia were observed to be increased after ICH, especially around the hematoma and lasted until day 3 and there was a gradual decrease observed after day 7 and remained same until day 28. Astrocyte activation observed by Western blot analysis showed a gradual increase in GFAP protein from day 1 and lasted until day 28. In conclusion, we believe that cofilin plays an important role in the activation of microglial subsequently leading to neuroinflammation and motor deficits following ICH. Developing therapies against cofilin might provide novel alternatives for ICH therapy.

P12: **The Identification of Factor H-Binding Protein(s) in Burkholderia**

I. A. Syed, L. S. Nejedlik, C. L. Lambert, M. E. Woodman, M. Mulye, V. P. Ferreira, J. F. Huntley, and R. M. Wooten

Background: Melioidosis is caused by the encapsulated Gram-negative organism *Burkholderia pseudomalle*i (Bp). Due to its low LD50, high infectivity, and antibiotic resistance, Bp is considered a Tier 1 select agent and there is great interest in characterizing virulence factors that may be targets for novel therapeutic agents. Our lab has previously shown that unopsonized Bp is not efficiently cleared by macrophages or neutrophils.

Hypothesis: We hypothesize that Bp has inherent resistance to complement deposition, mediated by expression of outer membrane proteins (OMPs) that bind complement factor H (fH) to avoid clearance by host immune mechanisms.

Methods and Results: While Bp was efficiently cleared by neutrophils if they possessed a critical threshold of C3 deposition, normal human serum (NHS)-opsonized Bp displayed significantly less C3 deposition than serum-sensitive bacteria. Upon far-Western analysis of Bp OMPs for fH binding, it was determined Bp expresses a protein capable of binding fH of ~37kD. Identification of the specific protein(s) involved in fH binding via 2D gel electrophoresis is underway.

Conclusions: Bp is inherently resistant to C3 deposition, but can be efficiently cleared/killed by neutrophils if critical C3 levels are achieved. Bp expresses at least one protein on their outer membrane capable of binding fH.

Moving Forward: We are currently refining our 2D gel electrophoresis protocol for maximum efficiency, after which it will be used for far-Western and mass spectrometric analyses on spots corresponding to factor H binding.

P13: **Mini-GAGR, an intranasally applied polysaccharide, activates the neuronal Nrf2-mediated antioxidant defense system**.

Kelsey Murphy, Dong-Shik Kim, and Joshua Park

Oxidative stress triggers and exacerbates neurodegeneration in Alzheimer's disease (AD). Various antioxidants reduce oxidative stress, but these agents have little efficacy due to poor blood-brain barrier (BBB) permeability. Additionally, single-modal antioxidants are easily overwhelmed by global oxidative stress. Activating nuclear factor erythroid 2 (NF-E2)-related factor 2 (Nrf2) and its downstream antioxidant system are considered very effective for reducing global oxidative stress. Thus far, only a few BBB-permeable agents activate the Nrf2-dependent antioxidant system. Here, we discovered a BBB-bypassing Nrf2-activating polysaccharide that may attenuate AD pathogenesis. Mini-GAGR, a 0.7-kDa cleavage product of low-acyl gellan gum, increased the levels and activities of Nrf2-dependent antioxidant enzymes, decreased reactive oxygen species (ROS) under oxidative stress in mouse cortical neurons, and robustly protected mitochondria from oxidative insults. Moreover, mini-GAGR increased the nuclear localization and transcriptional activity of Nrf2 similarly to known Nrf2 activators. Mechanistically, mini-GAGR increased the dissociation of Nrf2 from its inhibitor, Kelch-like ECH-associated protein 1 (Keap1), and induced phosphorylation and nuclear translocation of Nrf2 in a protein kinase C (PKC)- and fibroblast growth factor receptor (FGFR1)-dependent manner. Finally, 20-day intranasal treatment of 3xTg-AD mice with 100 nmol of mini-GAGR increased nuclear p-Nrf2 and growth-associated protein 43 (GAP43) levels in hippocampal neurons, reduced p-tau and β-amyloid (Aβ) peptide-stained neurons, and improved memory. The BBB-bypassing Nrf2-activating polysaccharide reported here may be effective in reducing oxidative stress and neurodegeneration in AD.

P14: **Nitric Oxide's role in suppression of HER2- positive breast cancer via S-Nitrosylation**

Yashna Walia, and Saori Furuta

Breast cancer is the second most common cause of cancer-related deaths among women in the US. Even with great advancements in screening and diagnostic techniques, the mortality rate of breast cancer remains high. This consistently high mortality rate can be attributed to tumor heterogeneity, due to which identifying new treatments have become challenging. HER2, an oncogene is involved in approximately 30% of all breast cancer cases. Its overexpression plays an important role in development and progression of breast cancer. Previously, it has been identified that HER2 overexpression is due to gene amplification, but in recent studies it has been revealed that in rare cases, this overexpression is not dependent on gene amplification. Therefore, there is a critical need to elucidate the unexplored non-genetic process that leads to HER2 overexpression. We theorized that changes in micro-environment, such as aberrant nitric oxide (NO) production, is at least in part involved in HER2 overexpression. We also aimed to successfully restore the physiological NO level in order to ascertain the effect of the restoration on HER2 expression and malignant characteristics. Our project reveals a link between aberrant NO production and HER2 overexpression. Our results also provides a novel therapeutic potential of restoring the NO level in the treatment of breast cancer**.**

P15: **Serum Deoxyribonuclease I Activity as a Latent Biomarker of Liver Cancer**

Rachel M. Golonka, Beng San Yeoh, Jessica L. Petrick, Stephanie J. Weinstein, Demetrius Albanes, Andrew T. Gewirtz, Katherine A. McGlynn, and Matam Vijay-Kumar

Deoxyribonuclease I (DNase I) is an enzyme that degrades extracellular DNA and thus, has been exploited as an anti-cancer agent and a cancer diagnostic marker. Yet, whether DNase I could be utilized as a latent biomarker for cancer is unknown. To ascertain its predictive value, we employed dysbiotic toll-like receptor 5 deficient (T5KO) mice, which develop liver cancer upon feeding the fermentable fiber, inulin. Post one week of inulin feeding, serum DNase I activity was measured in the T5KO mice. Surprisingly, only a subset (~40%) of T5KO mice exhibited increased DNase I activity. Intriguingly, only the subset of T5KO mice with high DNase I activity developed liver cancer after 6 months of inulin feeding, suggesting that DNase I activity could foretell liver cancer development. To investigate its human relevance, we measured human sera that were collected at baseline (8.6–21.2 years before liver cancer development) in the Alpha-Tocopherol Beta-Carotene (ATBC) study. The final analytic cohort included 224 primary liver cancer cases and 224 age- and sex-matched controls. Using multivariable-adjusted conditional logistic regression, we observed that DNase I activity in the highest quartile was significantly associated ($p < 0.01$) with a greater than three-fold risk of developing liver cancer (DNase I activity >2.72 units/mL, Hazard Ratio (HR) = 3.30, 95% Confidence Interval (CI) = 1.64 to 6.65). Overall, this study unravels the potential for DNase I activity to be a latent biomarker for liver cancer. Moreover, the parallel DNase I results between mouse and human emphasizes the translational potential of our novel, preclinical diet-induced liver cancer model.

P16: **Paraoxonase Regulation of Cardiotonic Steroids in Chronic Kidney Disease**

Chrysan J. Mohammed, Subhanwita Ghosh, Bruce S. Levison, Yanmei Xi, Pamela S. Brewster, Andrew L. Kleinhenz, Erin L. Crawford, Deepak Malhotra, Richard W. James, Philip A. Kalra, Steven T. Haller, and David J. Kennedy

The 2-pyrone ring structure of cardiotonic steroids (CTS) is critical for their binding to the Na+/K+-ATPase and initiation of pro-fibrotic signaling in chronic kidney disease (CKD). Paraoxonases (PONs) are enzymes capable of hydrolyzing compounds similar to the 2-pyrone rings in CTS, however the native physiologic substrate(s) of PON's are unknown.

Hypothesis: 2-pyrone containing CTS are substrates for PON hydrolytic activity and this specific activity is decreased in CKD.

Methods/ Results: We examined the ability of the CTS to compete with a chemically similar specific fluorogenic substrate of PON's (7-hydroxycoumarin). Purified RR and QQ genotype of PON-1 was reacted with 7-hydroxycoumarin in the presence and absence of CTS. PON-1 hydrolytic activity toward 7-hydroxycoumarin was significantly reduced in the presence of CTS for both genotypes. To confirm that this reduction was related to hydrolysis of CTS, a specific LC-MS assay was developed to measure the 2-pyrone active form of CTS. Incubation of CTS with PON-1 overexpressing cells showed a significant decrease in the 2-pyrone form of CTS at 24 hours. We measured circulating PON-1 protein (ELISA) and 2-pyronase-like activity in diabetic nephropathy CKD patients vs non-CKD controls. We found that while circulating PON-1 protein levels was not significantly increased across CKD stages, circulating PON-1 2-pyronase-like activity was significantly decreased across all CKD stages vs non-CKD controls.

Conclusion: CTS may be physiologic substrates for PON's and participate in a novel regulatory mechanism via hydrolysis of the CTS 2-pyrone ring. Furthermore, circulating PON-1 appears to have diminished 2-pyronase-like activity in the setting of CKD.

P17: **Characterizing regulators of the alternative pathway of complement in inflammatory disease**

Sara R. Moore, Sean A. Ehinger, Janet S. Lee, William Bain, Rick van der Geest, Gowthami M. Arepally, and Viviana P. Ferreira

The complement system is a central component of innate immunity. It consists of three pathways (classical, lectin and alternative) that result in pro-inflammatory outcomes including cell lysis, proinflammatory mediator production, opsonization, and antigen processing. The alternative pathway (AP) initiates spontaneously on any cell that cannot regulate it and also amplifies the classical and lectin pathways. Thus, tight regulation is required for effective, normal AP function. Factor H negatively regulates the AP by preventing activation on host cells and in the fluid phase. Alternatively, properdin is the only positive regulator of complement that initiates and stabilizes the AP. Interestingly, properdin normally circulates in blood as dimers, trimers and tetramers in a 1:2:1 ratio, with tetramers being more active than the other oligomers. It is unknown whether oligomer distribution changes during chronic inflammatory disease as a way of potentially modulating inflammation. We developed a novel ELISA-based assay that measures the function of properdin and the readout correlates with the presence of more tetramers versus other polymers. Using this assay, the assessment of properdin function in serum from patients with sepsis or coagulopathies is underway. Additionally, the concentration of properdin, Factor H and Factor B (an AP protein) were measured by ELISA, and all data is being compared to overall AP activity (hemolysis assay) in order to better understand how the composition of key regulators contributes to AP activity in inflammatory disease. These studies will contribute to understanding the role of complement in pathological inflammation, with potential applications for developing anti-inflammatory treatments.

P18: **Targeting polyamine metabolism in Enzalutamide-resistant prostate cancer**

Sayani Bhattacharjee, Rebecca Wynn, and Nagalakshmi Nadiminty

Prostate cancer is one of the most common non-skin male-related cancers in the world and has a 30% 5-year survival rate for when the cancer has spread to other parts of the body. In most cases of advanced prostate cancer treatment, the patient develops resistance to the therapy after some time, thus creating a dire need for establishing newer targets and approaches for the management of therapy resistant prostate cancer. Enzalutamide is an antiandrogen used for the treatment of castration-resistant prostate cancer. However, resistance to enzalutamide develops in most patients within 9-15 months. Using Enzalutamide-resistant cells, our studies aimed to reveal novel pathways that contribute to the development of therapy resistance. We found that the expression levels of polyamine metabolism enzymes were higher in Enzalutamide-resistant prostate cancer cells compared with parental cells. Cell survival assay and clonogenic assays showed that a polyamine analog, DENSPM (Diethyl norspermine), was more effective against Enzalutamide-resistant cells in comparison with parental cells. In addition, treatment with the combination of DENSPM and Enzalutamide re-sensitized the resistant cells to Enzalutamide. These data show that polyamine metabolism might be one of the pathways through which prostate cancer cells acquire resistance to Enzalutamide. Moreover, we also found that a combination of DENSPM and PARP inhibitors could abolish Enzalutamide-resistant cell survival. Thus, polyamine metabolism may also play an important role in the mechanism of action of PARP inhibitors. These findings indicate that disturbing polyamine metabolism using analogs such as DENSPM may represent an effective therapeutic strategy against Enzalutamide-resistant prostate cancer.

P19: **Assessment of function of neutrophil-derived properdin: identification of a novel form of complement regulation**

Smrithi S. Menon, Sean Ehinger, Neeti S. Galwankar and Viviana P. Ferreira

The complement alternative pathway (AP), essential for innate immunity, enhances diverse inflammatory responses. The AP is tightly regulated and properdin is the only known positive regulator. It stabilizes the needed AP enzymatic convertases and also initiates the AP. Properdin circulates in blood as head-to-tail associations of non-functional monomers, forming dimers, trimers and tetramers in a 1:2:1 ratio, with increased function for tetramers followed by trimers and dimers. Highly active non-physiological aggregates form when purified properdin is repeatedly freeze/thawed. Activated neutrophils are the main source of properdin, which is stored in secondary granules and are released into the surrounding microenvironment. The distribution of secreted properdin oligomers by neutrophils and whether this distribution modulates local inflammation are unknown. We generated a novel ELISA-based assay that measures properdin function that correlates with the presence of higher-order properdin oligomers. Using this assay, we determined that neutrophil-derived properdin had undetectable function, even though its function in a standard hemolysis-based functional assay, which is not affected by change in oligomer distribution, was unaltered. The lack of function detected by our ELISA may be due to properdin being released as non-functional monomers that later polymerize in blood, as a way of controlling strong local complement activation. To assess this, we repeatedly freeze/thawed the neutrophil supernatant to promote properdin oligomerization (as occurs with pure properdin from plasma) and functional analysis of this properdin is under way. Our study, by delineating the distribution of neutrophil-derived properdin forms, will contribute to understanding molecular mechanisms of AP regulation in inflammatory microenvironments.

P20: **Targeted disruption of Na/K-ATPase- Src signaling cascade significantly reduces renal inflammation and oxidative stress following 5/6th partial nephrectomy**

Fatimah K. Khalaf, Prabhatchandra Dube, Andrew L. Kleinhenz, Deepak Malhotra, Amira Gohara, Christopher A. Drummond, Jiang Tian, Steven T. Haller, Zijian Xie, and David J. Kennedy

Objective: Cardiotonic steroids are Na/K-ATPase alpha-1 (NKA α-1) ligands that are increased in volume expanded states such as chronic kidney disease (CKD). We have shown that NKA α-1- Src signaling pathway is essential mediator of cardiotonic steroid induced inflammation in renal epithelial and immune cells. As inflammation and oxidative stress play a central role in the onset and progression of renal injury associated with CKD, we performed 5/6th partial nephrectomy (PNx) to induce CKD and study the role of the NKA α-1and Src kinase in mediating renal inflammation and oxidative stress.

Methods/ Results: First, to examine the role of the NKA α-1 in mediating PNx induced renal inflammation and oxidative stress, wild type and NKA α-1+/- mice were subjected to either 5/6th partial nephrectomy or sham surgery. Six-weeks post PNx surgery, kidneys were sectioned and stained for 8-OHdG. We found that while PNx induced oxidative stress in the wild type kidneys, NKA α-1+/- mice demonstrated significantly lower levels of DNA oxidation. Twenty-four hour urine from the NKA α-1+/- mice showed significantly lower excretion levels of 8-OHdG compared to wild type controls. Histological examination demonstrated that NKA α-1+/- mice had significantly lower inflammation levels compared to wild type mice. Additionally, we examined recruitment of CD68 positive immune cells within the kidney. Kidneys from NKA α-1+/- mice showed less macrophage infiltration compared to kidneys from the wild type controls.

Conclusion: NKA α-1-Src kinase complex plays central role in regulating the renal inflammatory response in the setting of CKD.

P21 **Targeting the mDia formin-assembled cytoskeleton is an effective anti-invasion strategy in adult high-grade glioma patient-derived neurospheres**

Kathryn N. Becker, Krista M. Pettee, Arthur S. Alberts, Kevin A. Reinard, Jason L. Schroeder, and Kathryn M. Eisenmann

High-Grade Glioma (HGG, WHO Grade III-IV) accounts for the majority of adult primary malignant brain tumors. Failure of current therapies to target invasive glioma cells partly explains the minimal survival advantages: invasive tumors lack easily-defined surgical margins, and are inherently more chemo- and radioresistant. Much work centers upon Rho GTPase-mediated glioma invasion, yet downstream Rho effector roles are poorly understood and represent potential therapeutic targets. Roles for the mammalian Diaphanous (mDia)-related formin family of Rho effectors have emerged in invasive/metastatic disease. mDias assemble linear F-actin to promote protrusive cytoskeletal structures underlying tumor cell invasion. Small molecule mDia intramimic (IMM) agonists induced mDia functional activities including F-actin polymerization. mDia agonism inhibited polarized migration in Glioblastoma (WHO Grade IV) cells in threedimensional (3D) in vitro and rat brain slice models. Here, we evaluate whether clinicallyrelevant high-grade glioma patient-derived neurosphere invasion is sensitive to formin agonism. Surgical HGG samples were dissociated, briefly grown as monolayers, and spontaneously formed non-adherent neurospheres. IMM treatment dramatically inhibited HGG patient neurosphere invasion both at neurosphere embedding and mid-invasion assay, inducing an amoeboid morphology in neurosphere edge cells, while inhibiting actin- and tubulin-enriched tumor microtube formation. Thus, mDia agonism effectively disrupts multiple aspects of patient-derived HGG neurosphere invasion.

P22: **The Role of 14-3-3ζ in IL-17A Signaling**

Cara Peter and Ritu Chakravarti

Large Vessel Vasculitis (LVV) is a family of autoimmune diseases that affect large vessels, including the aorta and its branches. Like other autoimmune diseases, several cytokines including IL-17A contribute to the pathogenesis of LVV. Our group previously found that 14-3-3ζ is an autoantigen in LVV and is present in excess around the necrotic tissues in smooth muscle cells and infiltrating immune cells of aortic tissue with aneurysm. We questioned the role of 14-3-3ζ in the pathogenesis of disease and cytokine signaling. 14-3-3ζ is a cytosolic protein with known knowledge of its involvement in several cellular pathways, however its involvement in immune signaling has not been reported before. Using human and murine cell lines, we determined that 14-3-3ζ promotes IL-17A signaling in several cell types. We also observed that 14-3-3ζ interaction with members of the TRAF family of proteins, is responsible for its role in IL-17A signaling. Thus, we hypothesize that 14-3-3ζ is the key component of Il-17A signaling and LVV pathogenesis.

P23: **Role of cofilin in age-related neuroinflammation: In vivo studies**

Amsha S. Alsegiani, Saleh I. Alaqel, and Zahoor A. Shah

Our study aims to define the role of cofilin in age-related neuro-inflammation. Cofilin protein, the major ADF/cofilin isoform, plays an essential role in severs and depolymerizes actin filaments to generate the dynamics of the actin cytoskeleton. We observed that cofilin is upregulated in neuroinflammatory conditions and thus, it might also play a role in age-related neuroinflammation. We are interested, in particular, to compare the microglial cofilin expression in the brain between aged and young mice, as well as mice with induced neuro-inflammation compared with wild-type mice.

Our result showed an increase in expression level of cofilin in the aged mice compared to the young mice and in mice with induced neuro-inflammation compared to the wild-type mice. However, one interesting observation made from our study is that the young mice that were injected with lipopolysaccharide (LPS) did not show as high cofilin levels in their microglia compared with aged wild-type mice. Interestingly, these results are also consistent with the results obtained from our in-vitro study where we used a human microglia HMC3 cell line. Hence, we are interested in performing further investigations to understand the potential role of cofilin in age-related inflammation, which would define it as a potential biomarker in neuroinflammation.

P24: **Increased Circulating Levels of Mitochondrial Fragments Leads to Innate Immune System Activation Prior to the Onset of Hypertension in Dahl Salt Sensitive Rats**

Jonnelle Edwards, Sarah Galla, Cameron G. McCarthy, Nicole Bearss, Shaunak Roy, Bina Joe, and Camilla F. Wenceslau.

Aims**:** Chronic activation of the immune system contributes to kidney injury and hypertension. Mitochondria carry hallmarks of their bacterial ancestry and thus have emerged as a significant source of inflammatogenic damage-associated molecular patterns. One of these hallmarks is that they still use formylated peptides (NFPs) as an initiator of protein synthesis. NFPs activate formyl peptide receptor (FPR), a G-protein coupled receptor, and leads to receptor internalization and desensitization. We have observed that mitochondrial NFPs are elevated in the circulation of spontaneously hypertensive rats (SHR) and formyl peptide receptor (FPR) blockage decreases blood pressure in this strain. Also, we and others observed that FPR downregulates upon activation in immune and non-immune cells. Given that cell death and gut dysbiosis are present in salt sensitive hypertension, we hypothesized that Dahl sensitive rats would have decreased FPR expression, and that the decrease could be attributed to increased plasma levels of circulating mitochondria and/or bacteria NFPs from the cell death and leaky gut, respectively, and contributes to hypertension. Sex differences were also investigated.

Methods**:** Male and Female Dahl Sensitive (S) and Resistant (R) rats (6-week old) were given a low or high salt diet (2% NaCl) for 38 days. Blood pressure (BP) was measured by telemetry. After the 3-day recovery period from the telemetry surgery, the systolic blood pressures (SBP) were taken, and the rats were grouped. Rats received normal drinking water or water supplemented with neomycin (0.5 g/l, GIBCO) for 3 weeks. Kidney samples were taken from all rats and used for mRNA extraction and purification. The mRNA was made into cDNA to test gene expression for FPR. Blood was collected to measure mitochondrial NFPs. T-test *$p<0.05$; n= 8-11 for Dahl S and R; n=7 for Dahl + neomycin.

Results and Conclusion**:** FPR expression was downregulated 4-fold from male and female Dahl S when compared to Dahl R (AU: FPR mRNA: Dahl R 4.5 ± 1.3 vs. Dahl S: 0.7 ± 0.2*) (Figure 1). This phenomenon was independent of salt and sex differences. Antibiotic treatment partially restored FPR expression from Dahl S animals when compared to Dahl S treated with neomycin (AU FPR mRNA: Dahl S 4.5 ± 1.3 vs. Dahl S-neomycin treated: 2.74 ± 0.99*). ND6, a mitochondrial protein, is 1.7-fold higher in plasma from Dahl S compared to Dahl R. Unexpected, salt diet did not change this parameter. We have observed for the first time that FPR is downregulated in Dahl animals independent of salt probably due to overstimulation of this receptor. Therefore, FPR activation due to increased levels of bacterial (NFPs) could be associated with the development of renal injury at an early age independently of salt and elevations in arterial pressure.

P25: **Properdin inhibition prevents complement-mediated lysis of red blood cells that cannot regulate complement activation**

Jin Y. Chen, Neeti S. Galwankar, Heather N. Emch, Samuel A. Merrill, Robert A. Brodsky, and Viviana P. Ferreira

Complement, a tightly regulated system, central to innate immunity, is composed of the alternative (AP), classical and lectin pathways. Consequences include cell lysis and proinflammatory mediator production, among others. Altered complement regulation can lead to paroxysmal nocturnal hemoglobinuria (PNH) or atypical hemolytic uremic syndrome (aHUS), two hematological diseases. Patient red blood cells (RBCs) succumb to varying degrees of AP-mediated lysis. Soliris, a monoclonal antibody (MoAb) that inhibits the end stages of complement, is the only FDA-approved drug for these diseases and is incompletely effective. Here, we aimed to understand the role of properdin in these diseases by comparing complement inhibitors (Soliris, C3 inhibitor Cp20, and AP inhibitory MoAbs against Factor B (FB) and properdin) in their ability to prevent complement-mediated lysis of RBCs with defective complement regulation. In *in vitro* PNH-like and aHUS-like hemolysis assays, where complement regulation is blocked on normal RBCs, properdin and FB inhibition completely protected the RBCs and properdin inhibition was significantly more effective than anti-FB, Cp20, and Soliris as measured by IC_{50} concentrations (~15-fold, 140-fold, and 4 to 12-fold higher, respectively). However, Soliris did not protect ~40% of the cells in the PNH model. Moreover, when tested with RBCs from PNH patients, the results and comparisons were similar for all inhibitors, except for Soliris where the IC_{50} could not be determined (>22-fold the anti-properdin MoAb IC_{50}). Altogether, the data indicate properdin inhibition is the most efficient in aHUS and PNH models. Further studies aimed at evaluating the effect of inhibiting properdin in diseases are underway.

P26: **A Bioinformatics Pipeline for Nuclear Hormone Receptor Analysis**

Scott Miruzzi, Darren Gordon, Terry Hinds, and Robert McCullumsmith

Nuclear hormone receptors (NHRs) are a complicated but significant component of gene transcription, playing a large role in whether or not genes get transcribed and under what conditions transcription occurs. Coregulators will respond to certain queues such as ligands binding to the NHR to regulate gene expression. Measuring the activity and characterizing the effects of certain coregulator activity or certain profiles of coregulators is of increasing interest as their role in diseases starts to be further explored. With the change in coregulators comes the change of gene expression, and the field of bioinformatics to analyze gene expression is also of increasing value in its own right. Here we will discuss merging a new NHR chip assay that characterizes coregulator activity with bioinformatics to create a pipeline for an easy and streamlined analysis of data. Having an established pipeline for data analysis of coregulators opens the door to understanding why gene expression differences exist in myriads of conditions, enables the assessment of the impact of these changes on a genome-wide level and can be a gateway for further hypothesis-generating information.

P27: **Retinoic acid-induced upregulation of native anti-inflammatory pathways is a potential novel sepsis treatment**

Hallie H. Dolin, Xiaohuan Chen, and Zhixing K. Pan

The human immune system can eliminate many pathogens through the innate inflammatory process. However, loss of normal regulation can lead to disease. Sepsis, one of many such inflammatory diseases, is a major cause of in-hospital morbidity and mortality in the United States. It manifests as a systemic inflammatory state, symptoms of which can lead to shock. Sepsis consequently has a high mortality rate, averaging 30%. Artificial induction of endogenous anti-inflammatory molecules may be a viable therapeutic target for sepsis. Among the most important pro-inflammatory pathways in sepsis is mitogen-activated protein kinase (MAPK), which is integral to the inflammatory process of sepsis. MAP kinase phosphatase 1 (MKP-1) is an endogenous negative regulator of MAPK-induced pathways. Previous research has shown that MKP-1, when overexpressed, is protective against experimental sepsis. Conversely, knockout of MKP-1 leads to spontaneous inflammation. Using a novel drug-screening process, we have found that retinoic acid can significantly upregulate MKP-1 levels. In in vitro models, retinoic acid reduces levels of pro-inflammatory cytokines and intermediates downstream of MAPK. In addition, using a mouse model, we have found that retinoic acid significantly decreases mortality when administered with LPS, as compared to positive control mice. Liver function and lung architecture are also preserved in comparison to control mice. These results indicate that retinoic acid may be a viable novel sepsis treatment, with MKP-1 upregulation as a likely mechanism. This may expand the range of safe anti-inflammatory treatments for sepsis in the United States as well as in less-developed countries.

P28: **Unexpected phenotypic variation found in 8kb Dmp1cre mice questions future use of this model for targeted deletion of PPARα in osteocytes**

Amit Chougule, Sudipta Baroi, and Beata Lecka-Czernik

The nuclear receptor PPARα is a major regulator of energy production and lipid metabolism, however its role in bone homeostasis has not been analyzed in details. Previously, we observed that mice with global deletion of PPARα have decreased bone formation, increased bone resorption and increased marrow fat volume. Likewise, we have showed that sclerostin protein is under negative control of PPARα. Sclerostin, an inhibitor of Wnt signaling and anti-osteoanabolic protein, is mainly produced by osteocytes (OT) and is a pharmaceutical target to treat osteoporosis. Thus, we hypothesized that PPARα in OT plays an important role in regulation of bone and energy metabolism. To test this hypothesis, we developed OT specific PPARα knock-out, 8kb-Dmp1CrePPARαfl/fl, mouse (αOT-KO). DMP1 (Dentin Matrix Protein-1) is essential for bone mineralization and expressed specifically in OT. Several studies have demonstrated that 8kb-DmpCre is more specific than 10kb-DmpCre for OT genetic manipulation. We observed that αOT-KO mice have variable bone and metabolic phenotype, which prevented us to use this model for our studies. We traced these characteristics to 8kb-Dmp1Cre mice. Thus, 40% of 8kb-Dmp1Cre cohort had significantly reduced fat content, improved glucose tolerance, and high bone mass. Such heterogeneity was not observed in PPARαfl/fl or WT mice. Due to significant differences between 8kb-Dmp1Cre and PPARαfl/fl+WT controls, final results of αOT-KO study were confounding and highly dependent on controls selected for analysis. In summary, genetic background of 8kb-Dmp1Cre may have delivered heterogenous phenotype in these mice. Thus, we have developed an alternate OT specific mouse model, 10kb-Dmp1CrePPARαfl/fl, for future study.

P29: **Role of CXC4-Dependent LASP1-AGO2 Interaction in Breast Cancer**

Augustus M.C. Tilley, and Dayanidhi Raman

The goal of our research is to investigate the role of the CXCR4-LASP1 axis in modulating the activity of Argonaute2 (Ago2) in breast cancer. CXCR4 is primarily involved in metastasis, and elevated levels of CXCR4 predicts poorer survival in patients. CXCR4 induces tumor cell migration and invasion upon activation by its ligand CXCL12. We previously reported that CXCR4-mediated migration/invasion was ablated by stable knockdown of LIM and SH3 protein 1 (LASP1). To investigate potential mechanisms by which the CXCR4-LASP1 axis could mediate this phenotype, a proteomic analysis was performed, revealing components of the RNA-induced silencing complex as LASP1 interactors. It is possible that CXCR4 is altering the proteome through repression of translation or cleavage of mRNAs to facilitate tumor cell motility. Ago2 is a central component of the RNA-induced silencing complex and is a critical determinant of which mRNAs are repressed/degraded. Here we find that Ago2 directly binds to LASP1 through its LIM domain. The interaction of LASP1 with Ago2 was further validated by co-immunoprecipitation, GST-pulldown and proximity ligation assays. These results show that endogenous LASP1 is capable of interacting with Ago2 both basally and upon stimulation with CXCL12. Interestingly, Ago2 prefers to interact with a specific phosphorylation species of LASP1. LASP1 has two primary phosphorylation sites, Y171 and S146. Here we reveal that Ago2 preferentially interacts with a phospho-null S146 mutant and a phospho-mimetic Y171 mutant of LASP1. In order to discern the functional consequences of this interaction we will employ the dominant-negative and the dominant active phospho-mutants of LASP1. Once we have identified the functional consequences of this interaction, our goal is to disrupt it with small molecule inhibitors. Translationally, we hope the CXCR4-LASP1-Ago2 axis could be a novel and valuable molecular target for chemotherapy.

P30: **Altruistic drug design: a precision therapy platform that sacrifices earning potential in order to expedite translational timelines**

Justin Creeden, Shi-He Liu, and Francis Charles Brunicardi

Here, we outline a novel platform for drug development that expedites the translational process and increases the probability of successfully carrying a safe and effective drug through clinical trials. Advantages and disadvantages of this platform are discussed and its use in the creation of the “Combination-3” (C3) formulation is examined as proof of concept.

P31: **Spexin Differentially Regulates Adipogenesis in Brown and White Adipose Tissue Depots**

Shermel B. Sherman, Riley Powers, Ashima Thusu, and Jennifer Wootton Hill

In the last decade, neuropeptide Q or spexin (SPX) was implicated in central and peripheral functions including feeding suppression and lipid metabolism. To test whether spexin acts on brown and white adipose tissue, we performed subcutaneous micro-osmotic infusion of spexin in mice with diet-induced obesity. Our preliminary results indicate that spexin may differentially regulate biomarkers of adipogenesis and insulin sensitivity in brown and white adipose tissue. Spexin infusion increased interscapular brown adipose tissue mass and mRNA expression of adiponectin, fatty-acid binding protein 4, lipoprotein lipase, and C/EBP-α in female C57BL/6 mice. Within gonadal white adipose tissue, spexin and PPAR-γ2 mRNA expression was upregulated while immunohistochemistry of gWAT revealed that β3-adrenergic receptor expression was significantly decreased while adipocyte diameter was increased. These results indicate that spexin infusion differentially regulates adipogenesis within brown adipose tissue by increasing BAT mass and increasing non-adrenergic regulation of adipogenesis within gonadal white adipose tissue.

Oral Presentation Abstracts

O1: **RKIP-RhoA axis suppresses breast cancer lymph node and lung metastasis by modulating chemokine receptor expression**

Gardiyawasam Kalpana, and Kam C Yeung

Tumor metastasis suppressors impede secondary tumor formation by inhibiting one or more steps of the metastasis cascade without stimulating primary tumor growth. Raf-1 kinase inhibitor protein (RKIP) is a metastasis suppressor that inhibits metastasis in breast, prostate and several other types of cancers. The molecular mechanism through which RKIP executes its anti-metastasis effects is not yet completely defined. The objective of the current study is to understand how RKIP inhibits breast cancer cell invasion and metastasis at the molecular level. Given their primary functions in actin cytoskeleton dynamics and cell movement regulation, Rho GTPases were studied as possible downstream effectors of RKIP. Among all Rho GTPases, RhoA has both pro-metastatic and anti-metastatic cell-context dependent functions. In our present study, we demonstrate that the increased anti-metastatic activity of RhoA is one of the causes of RKIP-mediated suppression of breast cancer metastasis. Upon RNAi-mediated RhoA gene knockdown, more tumor cells were detected in the sentinel lymph node and more colonies in the lungs, suggesting that RhoA suppresses breast cancer lymph node and lung metastasis. Further characterizations showed that RhoA knockdown primary tumors have a different chemokine receptor expression profile than that of the control tumors, thereby altering the chemotactic abilities of these cells, which caused the different metastatic potential of these breast cancer cells.

O2: **Hepatic injury associated with reduced urinary excretion and altered hepatic signaling pathways following chronic low dose exposure to microcystin-LR in a murine model of non-alcoholic fatty liver disease**

Apurva Lad, Robin Su, Joshua Briedenbach, Nicholas Carruthers, Paul Stemmer, Dilrukshika Palagama, David Baliu-Rodriguez, Dragan Isailovic, Andrew L. Kleinhenz, Fatimah K. Khalaf, Shungang Zhang, Prabhatchandra Dube, Chrysan Mohammed, Amira Gohara, Deepak Malhotra, Steven T. Haller, and David J. Kennedy

Cyanotoxins, like microcystins (MCs), are produced by cyanobacteria and pose a serious growing global public health risk. While current exposure guidelines have been extrapolated to humans based on studies performed in healthy animal models, their effect in at-risk populations with pre-existing liver disease is unknown. Hence, we hypothesize that the No Observed Adverse Effect Level (NOAEL) of MCs established in healthy mice would cause demonstrable hepatic injury in a murine model of Non-alcoholic Fatty Liver Disease (NAFLD). Ten-week-old male Leprdb/J and C57BL/6 mice were orally gavaged with 50μg/kg or 100μg/kg microcystin-LR (MC-LR, one of the most common microcystin congeners) or vehicle every 48 hours for 4 weeks. During the treatment, we observed a non-statistically significant trend in decreased survival in Leprdb/J mice whereas no deaths were observed with healthy C57BL/6 mice. We observed significant increase in histopathologic evidence of hepatic injury and hepatotoxic gene expression with MC-LR exposure in the Leprdb/J mice. Quantification of MC-LR levels in plasma and urine samples using LC-MS/MS method revealed measurable (~0.5 ng/mL) levels of MC-LR even 72 hours after administration in the Leprdb/J NAFLD model whereas the levels were undetectable in the healthy C57BL/6 mice. Furthermore Leprdb/J mice exhibited urinary excretion of MC-LR >60 times less than that of healthy C57BL/6 mice ($p<0.01$). Finally, phosphoproteomic analysis with mass spectrometry-based quantification of TiO2-enriched liver samples showed significant treatment-dependent altered phosphorylation patterns of key signaling pathways. Thus, our results suggest that the NOAEL of MC-LR results in significant hepatic injury in the setting of NAFLD.

O3: **Trichloroacetate, Dichloroacetate and Mixtures-Induced Developmental Toxicity in Zebrafish Embryos (Danio rerio)**

Omar N. Issa, Ezdihar A. Hassoun, and Frederick E. Williams

Water chlorination is a process used to eradicate bacteria and other waterborne pathogens. A major consequence is the generation of a class of compounds that is known as disinfectant by-products (DBPs). DBPs are two classes; halogenated and non-halogenated. TCA and DCA are the major halogenated compounds with concentrations of 130 and 63.1-133µg/L, respectively. Here, the developmental toxicity of TCA, DCA, and their mixtures were investigated in zebrafish (Danio rerio) embryos. Embryonic exposure to solutions of DCA (32mM), TCA (24 and 32mM), Mixture-I, and Mixture-II occurred at 4hpf and ended at 144hpf for percent death while at 80hpf for mixture's developmental landmarks. The CSE produced by 24mM TCA and Mixture-I was insignificant when compared with controls and each other. Alternatively, DCA induced YSE and hatching delay when compared with controls and other groups while TCA and Mixture-I were similar to controls. On the contrary, 32mM TCA and Mixture-II produced significant death at both 80 and 144hpf. Although the 32mM concentrations induced elevation in CSE in all exposure groups, the increases in CSE among TCA and Mixture-II groups were insignificant when compared with each other yet they were still significantly greater than those of DCA groups. Significant increases in YSE in all groups when compared with their corresponding controls but insignificant when compared across. All groups exposed to 32mM concentrations induced hatching delay which was significant when compared to controls yet insignificant with each other. The effect of all exposure groups on HR was shown to be insignificant except that of DCA.

O4: **Amoxicillin-responsive alterations in commensal gut microbiota are associated with lowering of blood pressure in young hypertensive rats**

Sarah Galla, Saroj Chakraborty, Xi Cheng, Ji-Youn Yeo, Blair Mell, and Bina Joe

Pediatric hypertension is recognized as an emerging global health concern. While new guidelines are developed for facilitating clinical management, the reasons for the prevalence of hypertension in children remain unknown. We hypothesized that, similar to the known link between gut microbiota and adult hypertensives, alterations in gut microbiota that occur very early in childhood, or in the mother's gut prior to birth, are also associated with an increased prevalence of pediatric hypertension. To test this hypothesis, we administered amoxicillin, the most commonly prescribed antibiotic to pediatric patients, to alter gut microbiota of just-weaned, genetically hypertensive Dahl Salt-Sensitive (S) rats and to pregnant S rats. Amoxicillin-treated rats had decreased BP compared to controls, and pups born to amoxicillin-treated rats also had reduced BP when started on a high salt diet. In the young rats treated with amoxicillin, the lowering effect on blood pressure persisted even after the antibiotics were discontinued. These changes in BP were associated with alterations in gut microbiota in response to amoxicillin treatment. Albeit in an experimental model, our results demonstrate the significant role of gut microbiota in the early development of hypertension and indicate that clearing dysbiotic gut microbiota early in childhood, or altering the maternal microbiota during gestation, may confer long-term benefits.

Funding for this work to BJ from the NHLBI/NIH (HL143082) is gratefully acknowledged.

O5: **Evolution of Recombination: RecBCD and AddAB in bacteria**

Deepti Gurung, and Robert M. Blumenthal

Restriction-modification systems (RMSs) are nearly ubiquitous among bacteria, and cut unprotected incoming DNA. Counterintuitively, evidence suggests that restriction can promote bacterial gene exchange. We hypothesize that the major recombinase determines the fate of DNA following restriction. RecBCD is the major recombinase among bacteria like E. coli, while others such as Bacillus subtilis use the related AddAB complex. The activity of these recombinases is controlled by short species-specific DNA sequences called Chi sites. Encountering a Chi site changes the recombinase from degradative to recombination mode. The hypothesis is that RMSs create entry points in DNA for RecBCD/AddAB, with positive or negative effects based on presence of Chi sites. To determine how broadly this hypothesis might apply, we examined the distribution of RecBCD and AddAB. These recombinases are sometimes misannotated, and it would be useful to find ways to distinguish them. We examined 129 bacterial species representing 30 phyla, using bioinformatics tools such as NCBI BlastP, MUSCLE, Weblogo, and Two-sample Logo analysis. We found that RecA is, as expected, highly conserved. Conservation curves revealed that RecBCD and AddAB are less conserved than RecA, but still widespread. Comparatively, RecC/AddB are less conserved than RecB/AddA, perhaps reflecting the RecC/AddB role as the subunit recognizing species-specific Chi sites. We were able to distinctly classify RecB vs. AddA and RecC vs. AddB in some bacteria where this had not previously been done. Interestingly, the Actinobacteria phylum is unusually diverse in its recombinase orthologs. Further phylogenetic studies will explore evolutionary relationships among their recombinases.

O6: **ASSESSMENT OF LIVER ENZYMES FOR THE DIAGNOSIS OF MICROCYSTIN INDUCED HEPATOTOXICITY IN THE SETTING OF PRE-EXISTING LIVER DISEASE**

Robin Su, Apurva Lad, Andrew Kleinhenz, Steven T. Haller, and David J. Kennedy

Objective: Microcystin-LR (MC-LR) is a liver toxin produced by cyanobacteria in Harmful Algal Blooms, which contaminate freshwater sources and have become a global health concern. There are currently no diagnostic methods for MC-LR induced hepatotoxicity. Alanine aminotransferase (ALT) and alkaline phosphatase (ALP) are common liver enzymes used clinically to assess liver disease and serve as potential diagnostic tools for MC-LR hepatotoxicity. Non-alcoholic fatty liver disease (NAFLD) is one of the most common forms of liver disease globally. In order to account for these individuals, who may be more susceptible to MC-LR toxicity, we assessed ALT and ALP for their sensitivity as diagnostic tools in murine and cellular models of NAFLD.

Methods/Results: In a Leprdb/J C57BL/6J mouse model of NAFLD, gene expression of ALT and ALP decreased dose-dependently after 4 weeks of MC-LR treatment (ALT $p<0.05$ in 100ug/kg group, ALP $p<0.01$ in 50ug/kg and 100ug/kg groups). No differences were found in circulating serum ALT or ALP levels among the treatment groups at 4 weeks.. In a HepG2 human liver cell model of NAFLD utilizing hyperglycemic conditions, gene expression of ALT decreased dose-dependently ($p<0.05$ at all dosage levels) and ALP was not found to change significantly. ALT and ALP intracellular and extracellular enzyme activities also did not change significantly with MC-LR treatment.

Conclusion: Based on in vivo and in vitro studies, we conclude that ALT and ALP are not sensitive or specific biomarkers for MC-LR induced hepatotoxicity in the setting of pre-existing liver disease.

O7: **Insulin receptor expressing T cell infiltration into the islets affects insulin producing beta cell functionality**

Neha D. Nandedkar, Emily L. Rhoades, Nirdesh Gupta, Swapnaa Balaji, Ali G. Al-Dieri, James Bretz, Hannah Hafner, Brigid Gregg, and Marcia F. McInerney

In human and the non-obese diabetic (NOD) mouse model, T cells have a major role in the autoimmune destruction of insulin producing pancreatic beta cells. Approximately 20% of the T cells from diabetic NOD mice have shown a high density of insulin receptor (IR) on their surface. When flow cytometry purified high density IR expressing or IR negative T cells from diabetic NOD mice were transferred into young irradiated non-diabetic NOD mice, only high density IR expressing T cell recipient mice developed insulitis and diabetes. This indicates that IR overexpressing T cells are capable of moving towards the source of insulin, that is the pancreatic beta cells in the islets. Our lab has developed a novel transgenic mouse model, C57BL/6-CD3FLAGmIR/mfm, in a non-diabetic background, genetically modified to express FLAG tagged mouse insulin receptor (mIR) on T cells to determine if IR expressing T cells have any role in diabetes development. These transgenic mice have shown evidence of IR overexpressing T cell trafficking into the pancreas. Although insulitis occurs in C57BL/6-CD3FLAGmIR mice, they were not hyperglycemic at any age. However, FLAG-mIR expressing T cell infiltration into the pancreatic islet in the transgenic mice affected beta cell functionality and caused metabolic abnormalities. Patients with diabetes as well as prediabetic NOD mice have shown similar irregularities. This study provides a novel mechanism involved in the initial progression of type 1 diabetes.

O8: **Performance of heterozygous dopamine transporter mice in a 5-choice serial reaction time test for studies of attention deficit hyperactivity disorder**

F. Resendiz Gutierrez. D. Muskiewicz, B. Patel, A. Simon O. Chaker, Y. Saber, and F.S. Hall

The 5-choice serial reaction time task (5-CSRTT) is used to study attention and impulsivity in mice. The test involves a specialized operant chamber in which 5 visual stimuli can be presented, which indicate which of 5 response (nose-pokes) will result in the presentation of a reinforcer. The mouse must attend to the appropriate stimulus in order to make a correct response (a measure of attention), avoid premature responses (a measure of impulsivity), or omissions (also a measure of attention). The procedure has been used to study a number of models of frontostriatal disorders in mice, including Attention Deficit Hyperactivity Disorder (ADHD). The aim of this study is to test performance in heterozygous Dopamine Transporter Knockout mice (DAT (+/-)) , a potential animal model of ADHD, with the ultimate aim of testing the effects of pharmacological treatments on attentional deficits. Methods: 4 Female DAT (+/-), 4 Female DAT (+/+), 4 Male DAT (+/-) and 3 Male DAT (+/+) were tested. After initial task performance is established, the subjects are tested for attentional impairments by varying the stimulus duration (SD), in order to identify conditions that are optimal (i.e. there are attentional impairments) for the testing of potential ADHD medications. Across 80 trials, stimulus duration is varied pseudo-randomly (16 trials each of 2, 1, 0.5, 0.25, 0.12, sec stimulus duration). SD is adjusted downwards so that performance is sub-optimal at the lowest durations. The goal is to establish a range of performance that degrades from optimal performance at high SD to sub-optimal performance at low and variable SD. Results: Female DAT (+/-) mice made more errors of omission than their (+/+) counterparts, but Male (+/-) mice made less than (+/+). There were no differences in number of premature or correct responses.

O09: **An investigation on the evolution of Mid-Range Inhomogeneous (MRI) sequences in the human genome**

Rajan Paudel, and Alexei Fedorov

Human genomic DNA has many regions with biased nucleotide composition (such as being unusually GC-rich, AT-rich, purine-rich on one strand, TG-rich, etc.).Those regions of sufficient length are called Mid-Range Inhomogeneity (MRI) sequences. MRIs occupy >5% of mammalian genome and, in some cases, have been associated with known biological functions(e.g., GC-rich MRIs include CpG islands that regulate expression of genes). We studied 70 million single-nucleotide polymorphisms(SNPs) from the Thousand Genome Database to understand the effect of mutations on the evolution of MRI regions. A majority of mutations inside MRIs either decrease or increase bias in their nucleotide composition. In other words, they are not neutral with respect to the MRI features, and their effects can be measured. We found that the point mutations in MRI regions preferentially degrade the nucleotide inhomogeneity, decreasing the biases in their nucleotide composition. The highest level of this mutational degradation was observed for the GC-rich MRIs. Degradation of MRI nucleotide bias is highest for novel mutations, and considerably lower for older mutations (those broadly widespread across populations). Thus, it appears as if some active selection process preserves the nucleotide compositional bias in all types of MRIs, which could explain how nucleotide bias is preserved in MRIs despite the intense influx of mutations during evolution, and suggests that each type of MRI inhomogeneity has(like the GC-rich MRIs) a biological role.

O10: **The novel thienopyrimidine derivative, RP-010, produces anti-prostate cancer efficacy by inducing the fragmentation of β-catenin**

Noor Hussein, Saloni Malla, and Amit.K.Tiwari

Thienopyrimidines are a versatile group of compounds that contain a biologically active pharmacophore and were reported to have anticancer efficacy in-vitro. Here, we report for the first time, that thieno[3,2-d]pyrimidine - based compounds, designated the RP series, have efficacy in prostate cancer cells. The lead compound, RP-010, was efficacious in PC3 and DU-145 prostate cancer (PC) cells (IC50< 1μM). The cytotoxicity of RP-010 was significantly lower in normal cells. RP-010 (0.5, 1, 2, and 4 μM) arrested prostate cancer cells in the G2 phase of the cell cycle, induced mitotic catastrophe and apoptotic signaling in both PC cell lines Mechanistic studies suggested that RP-010 (1 and 2 μM) inhibits the wingless-type MMTV (Wnt)/β-catenin signaling pathway, mainly by inducing β-catenin fragmentation, while down-regulating important proteins in the pathway, i.e. LRP-6, DVL3, and c-Myc. Interestingly, RP-010 (1 and 2 μM) induced the nuclear translocation of the negative feedback proteins, Naked 1 and Naked 2, in the signaling pathway. In addition, RP-010 (0.5, 1, 2, and 4 μM) significantly decreased the migration and invasiveness of PC cells in vitro. Finally, RP-010 did not produce significant toxic effects in zebrafish at concentrations up to 6 μM. In conclusion, RP-10 is a promising anticancer agent against metastatic prostate cancer and is safe in vivo zebrafish model. Future mechanistic and efficacy studies are needed in-vivo.

O11: **TDRD7 – a new antiviral effector of the interferon system**

Gayatri Subramanian, and Saurabh Chattopadhyay

The interferon (IFN) system is the first line of defense against virus infection. Pattern recognition receptors sense viral infections and trigger the synthesis of IFNs, which in turn amplify the synthesis of IFN stimulated genes (ISGs). The protein products encoded by ISGs carry out the antiviral effector function. ISGs inhibit one or multiple stages of virus life cycle to block viral replication. Furthermore, many viruses are dependent on cellular autophagy for their replication. This led us to hypothesize that some antiviral ISGs would inhibit autophagy pathway to block viral replication. To test this hypothesis, we screened a library of human ISG shRNA and isolated a novel antiviral ISG - tudor domain containing 7 (TDRD7). Our results indicate that TDRD7 inhibits autophagy by inhibiting the autophagy-initiating kinase AMPK. Genetic or chemical inhibition of AMPK restricts viral replication. We recently reported that TDRD7 can inhibit human pathogens such as Respiratory Syncytial Virus (RSV) and Parainfluenza Virus 3 (PIV3), from the Paramyxoviridae family. Studies are in progress to investigate the molecular mechanism of TDRD7's functions.

O12: **Coup TF-II role in SMAD signaling in renal fibrosis**

Usman M Ashraf, Vishnuprabu Durairajpandian, David J Kennedy, and Sivarajan Kumarasamy

The Chicken ovalbumin upstream promotor factor II (COUP-TFII) has a role as a transcriptional activator/repressor. In our previous studies, we have demonstrated that under a high salt (HS) regimen both the systolic and diastolic blood pressure (BP) of Coup-TFIImutant rats improved compared to Dahl Salt-Sensitive (SS) rats. The current study extended this investigation into understanding the mechanism by which a targeted disruption of Coup-TFII locus in SS rats leads to a renal protective phenotype. We saw that a pro-fibrotic protein TGF-β increase COUP-TFII expression in HK2 cells, suggesting that TGF-β is a positive regulator of COUP-TFII. In the COUP-TFII-/- HK2 stable cell line, we showed a down-regulation of SMAD2 and SMAD3 levels. In addition, we observed a down-regulation of both SMAD4 and SMAD7 levels in COUP-TFII-/- HK2 cells with a decrease in pro-fibrotic genes such has COL3A1. To validate our in-vitro findings, we measured the levels of renal fibrosis in SS and Coup-TFIImutant rats fed on HS diet. We saw that there was a significant increase in renal fibrosis, a decrease in creatinine excretion and a reduction in GFR seen in the Coup-TFIImutant rats. The molecular signaling in-vivo also demonstrated a down-regulation of SMAD2, SMAD3, and SMAD4 levels in Coup-TFIImutant rats while showing an up-regulation of Smad7. Pro-fibrotic genes such as Col1A1 and Col3A1 were also found to be down-regulated in Coup-TFIImutant rats. Overall, this study provides evidence that targeted disruption of COUP-TFII locus in SS rats showed a renal protective phenotype by regulating the SMAD signaling.