The Hormel Institute
University of Minnesota

2018-19 ANNUAL REPORT

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Accelerating ANSWERS to CANCER

MISSION STATEMENT

The mission of The Hormel Institute is to conduct research and provide education in the biological sciences with applications in medicine and agriculture. In pursuit of this mission, and as intended by its founders, The Hormel Institute generates fundamental knowledge and disseminates it to the scientific community worldwide. It also serves as a center of technical and educational expertise for the benefit of the Austin community, the surrounding region and the State of Minnesota.

MESSAGE FROM THE EXECUTIVE DIRECTOR

Today The Hormel Institute, University of Minnesota is a prominent cancer and chronic disease research center and high performing part of the Masonic Cancer Center.

After two major expansions in 11 years, The Hormel Institute is home to some of the world's expert cancer researchers, a stunning design for our cancer center, and labs filled with state of the art technologies.

We are focused on accelerating answers to cancer and other chronic diseases so people can live longer, healthier lives. The Hormel Institute is currently comprised of 140 faculty, researchers, and staff. We now have 19 research sections with plans to continue to fill the institute's labs with expert scientists to further our mission. In fact, two more research sections will be added in the late fall of 2019.

After joining The Hormel Institute in 1999, I have had a great and incredible journey with The Hormel Institute faculty and staff to help build The Hormel Institute, University of Minnesota, to where we are today.

Strengthened by leadership and support from the University of Minnesota, The Hormel Foundation, and our generous community, we are fully committed to enhancing and maintaining our quest for continued success through discoveries leading to cancer prevention and healthier controls.

I'm honored to serve as interim executive director and I thank you - on behalf of all of The Hormel Institute, UMN - for your continued support.

Sincerely,

Ann M. Bode, Ph.D.
Interim Executive Director

Structural Biology of Membrane Transport

Amer Alam, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

The Alam lab uses a combination of biochemical and structural (Cryo-EM and X-ray crystallography) techniques to primarily study macromolecules and macromolecular complexes involved in membrane transport, homeostasis, and biogenesis, with a particular focus on pinpointing lipid/protein interactions in context of membrane protein function. Maintenance of membrane bilayer integrity and tight control over material transfer across cellular and organellar membranes is central to proper physiological functioning. Dysfunction of these systems lies at the heart of several devastating, often fatal pathologies ranging from rare inherited diseases such as Zellweger's syndrome and adrenoleukodystrphy (both stemming from peroxisomal dysfunction) to a range of neurodegenerative disorders, diabetes, and cancer.

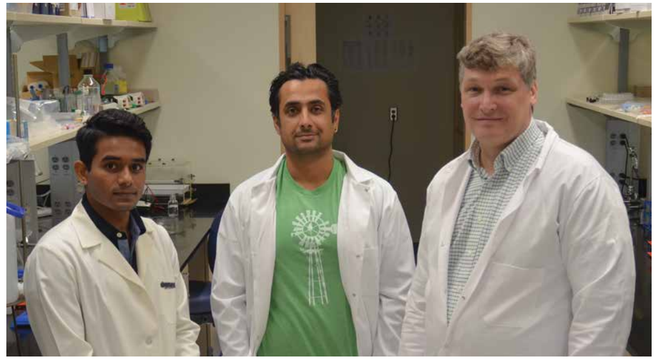
The Hormel Institute ranks amongst a handful of elite institutions housing a Titan Krios microscope combined with a direct electron detector, a setup designed to obtain near-atomic resolution (routinely achieving better than 0.5 nanometer resolution). This allows for accurate visualization of the molecular details underlying the functioning of macromolecules and has revolutionized structural biology and allowed for targeting of complex biological problems previously considered off-limits to high resolution visualization Such insight is invaluable in obtaining molecular details of membrane protein interactions with drugs, transport substrates, and inhibitors and can aid in the design of novel therapeutics targeting diseases stemming from membrane protein dysfunction.

Primary Research Areas:

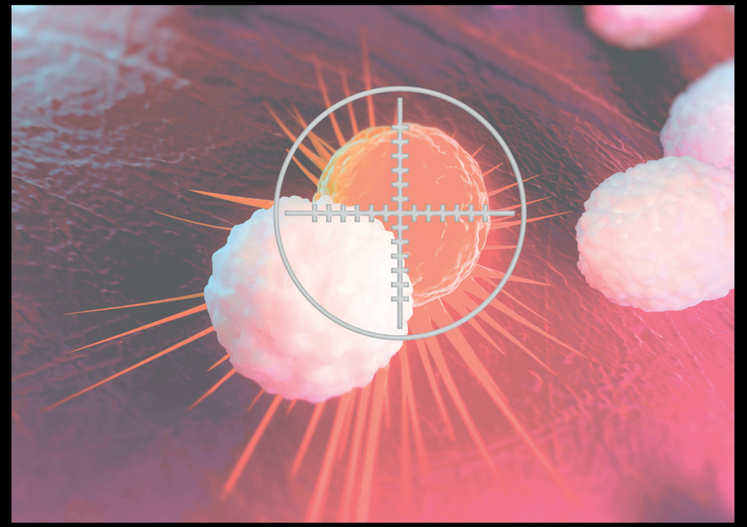
1) Membrane transport processes
2) Peroxisome biogenesis and homeostasis
3) Lipid/protein interactions
4) Lipid and fatty acid metabolism

Research Specialties:

1) Membrane protein biochemistry
2) Cryo electron microscopy
3) X-ray crystallography



FROM LEFT TO RIGHT:
Mehedi Hasan, Amer Alam, James Thompson



Publishing high impact research covering all aspects of precision oncology.

npj nature partner journals | npj Precision Oncology | The Hormel Institute University of Minnesota

npj PRECISION ONCOLOGY

- Springer Nature launched the journal *"npj Precision Oncology"* in partnership with The Hormel Institute, University of Minnesota in Austin, Minnesota, United States.
- *npj Precision Oncology*, is a new open access, international, peer-reviewed journal that publishes cutting edge scientific research in all aspects of precision oncology, from basic science to translational applications to clinical medicine.
- The journal defines precision oncology as cancer diagnosis, prevention, and/or treatment tailored specifically to the individual patient based on their genetic and/or molecular profile.
- *npj Precision Oncology* fills the need of a journal where both preclinical and early clinical studies can be published together to help support this rapidly advancing field.
- According to the Managing Editor at Nature Research "*Npj Precision Oncology* has been exceptionally successful". We have passed the first landmark of 25 peer-reviewed published articles, which makes our journal eligible to apply for inclusion in PUBMED, one of the first steps required to obtain an IMPACT FACTOR. We are also looking for 1-2 additional Associate Editors to bring on board.
- Featured topics include cancer development and prevention, therapies that target specific cellular pathways, cancer metabolism and genetic risk factors.
- In addition to publishing original basic science, translational and clinical research articles, *npj Precision Oncology* -also publishes case reports, brief communications, commentaries, perspectives, and review articles.
- The journal also publishes a professionally written Editorial Lay Summary to accompany each research Article, which summarizes the key issues being addressed within the article aimed to keep readership at the vanguard of new discoveries in the field.

Please visit us at: https://www.nature.com/npjprecisiononcology/

Molecular Bioengineering and Cancer Vaccine

George Aslanidi, Ph.D.

SECTION LEADER / ASSOCIATE PROFESSOR

"Our lab develops a safe and effective antigen-based vaccination strategy to activate patience immune system and redirect it to kill cancer cells."

George Aslanidi

Summary

We currently use our bioengineered capsid-optimized adeno-associated virus (AAV) vectors to develop anti-cancer vaccine. Briefly, tumor specific antigen carried by these novel AAV vectors are injected into animal model of melanoma or prostate cancer using standard needle vaccination procedures. As a result, local antigen presenting cells (APC) uploaded with the AAV-delivered tumor antigen either directly or by a cross-presentation pathway activate both a cytotoxic CD8+ T-cells and a humoral response against the tumor. We showed that our designed a AAV based vaccine has superior cytolytic capacity against encoded antigen. This vaccine prevents metastatic tumor spread, significantly delays solid tumor growth and synergized with emerging aPD1 therapy.

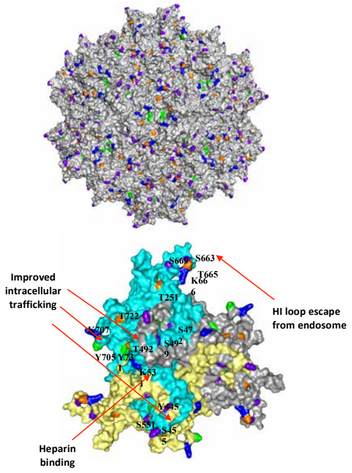
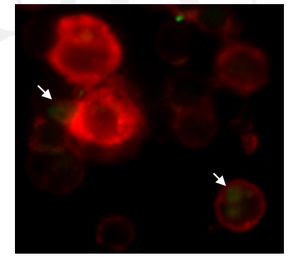


Figure 1: 3D structure of AAV 6 vectors.

Our research has demonstrated that a strong antigen-specific immune response is developed after a single injection of AAV-based vaccine in *in vivo* model. We attribute this to our novel capsid-optimized AAV based system, which has overcome many of the shortcomings of past viral cancer vaccine technologies: the precise modifications in the AAV capsid significantly increase transduction efficiency of the APCs and subsequent antigen presentation. In current studies we further optimized AAV expression cassette by fusing antigen with MHC class I molecule trafficking signals. This modification significantly increased AAV mediated level of both antigen-specific CD8+ T and CD4+ T cells, enhanced the formation of effector memory precursor (CD 62L-/CD127+) cells which ensure long-lasting anti-tumor immune response, as result, demonstrated superior Ag-specific killing capacity in *in vivo* model. We also were able to track down dendritic cell (DC) carrying reporter gene such as GFP in draining lymph nodes after *in vivo* model intramuscular injection and confirmed antigen expression by flow and microscopic analysis. More importantly, vaccination changes the immune landscape of the tumor by inducing massive invasion of immune cells, especially with CD8+ T cells and NK cells. Consequently, we showed that tumor-infiltrated lymphocytes displaying increased expression of PD-1 should sensitize tumor to aPD-1 therapy. Indeed, use AAV based vaccination in combination with aPD-1 treatment significantly delayed tumor development and extend *in vivo* model survival. Therefore, the path forward that we are planning to implement in our future studies to completely eradicate tumor, is multi-target vaccination in concert with other therapies that target tumor microenvironment and re-activate/support immune cells infiltrating tumor.

Additionally, we plan to test our anti-cancer vaccination approach in companion dogs with spontaneous melanoma. Importantly, companion dogs are exposed to the same environmental factors, and are developing cancer with clinical pathology very similar to humans. This makes dogs the most adequate preclinical model to evaluate the efficacy of our anti-cancer vaccination. Testing of our vaccine on clinically relevant dog patients will not only help to extend the life of people's best friends, but provide critical data ensuring progress into clinical application for humans is warranted.



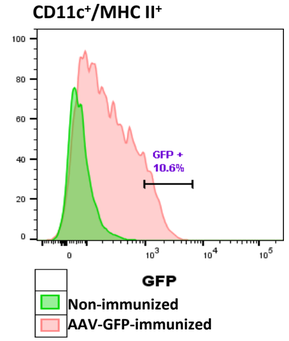
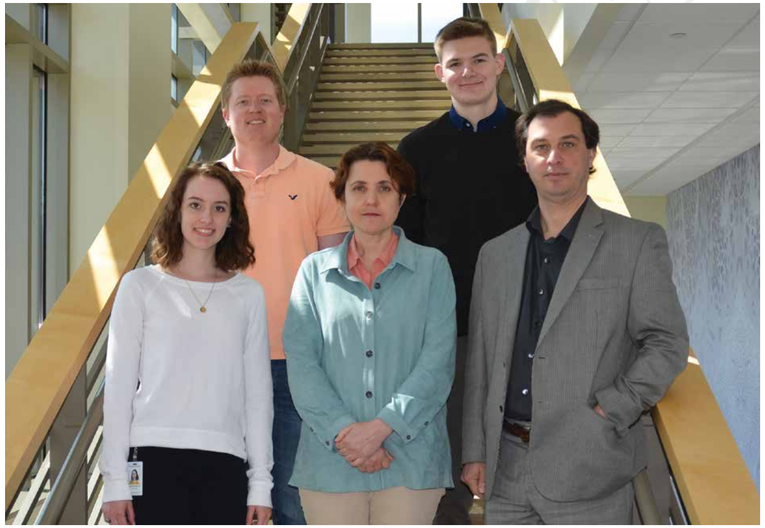


Figure 2: Bioengineered AAV 6 vectors infect Dendritic cell. Top Flow cytometry analysis, Bottom Microscopy analysis.

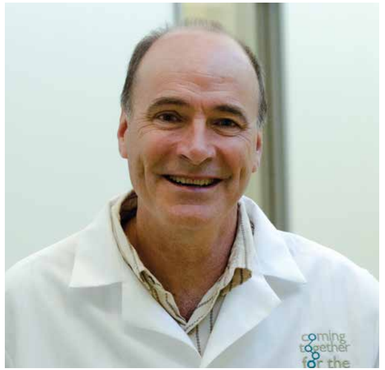


FROM LEFT TO RIGHT:
Front row: Kelly Vanhooser, Karina Krotova, George Aslanidi
Back row: Andrew Day, Noah Johnson

Membrane Biochemistry and Molecular Biophysics

Rhoderick Brown, Ph.D.

SECTION LEADER / PROFESSOR



Summary

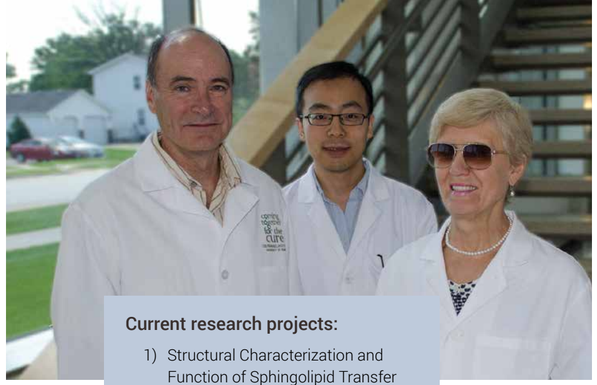
Our research focuses on proteins that interact with membrane lipids known as sphingolipids. Such proteins include sphingolipid transfer proteins that shuttle sphingolipids between intracellular membranes and protein lipid-binding domains (e.g. C2-domains) that act as targeting and anchoring devices for select membranes in cells. In membranes, certain sphingolipids form 'raft' microdomains that function as organizing regions for signaling kinases and target sites for certain viruses and bacteria. Early on, we investigated the physical basis for raft microdomain functionality by studying the structural features of sphingolipids that regulate their lateral and transbilayer interactions with other lipids in membranes. Our investigations relied on fluorescence spectroscopy, calorimetry, NMR, and Langmuir surface balance approaches. The latter approach, carried out in collaboration with the H.L. Brockman lab (UMN-Hormel Institute), enabled measurement of model membrane lateral elasticity which led to development of ways to quantitatively assess physical variations that occur within the 'raft environment' when the content and structure of sphingolipids and sterols change. This research identified physical features central to the lateral organizing functionality of sphingolipid-enriched microdomains.

We also study sphingolipid transfer proteins that help form and maintain 'raft' microdomains by shuttling sphingolipid between membranes in cells. These sphingolipid transfer proteins were initially identified as glycolipid transfer proteins (GLTPs) because of their specificity for glycosphingolipids. We performed the first cloning of GLTPs from various mammals (humans, bovine, porcine, mouse) as well as plants and fungi and developed bacterial expression systems to purify the proteins enabling determination of their molecular structures both in glycolipid-free form and complexed with different glycolipids by X-ray diffraction. Our findings revealed how GLTPs recognize and bind glycolipids to accomplish intermembrane transfer by shedding light on: i) GLTP binding site adaptation to accommodate different glycolipids; ii) the function played by intrinsic tryptophan residues in glycolipid binding and membrane interaction; iii) the structural basis for the more focused glycolipid selectivity of fungal GLTP and the GLTPH domain of human FAPP2. The findings are published in Nature, PLoS Biology, Structure, The Journal of Biological Chemistry, Biophysical Journal, Biochemistry, and Journal of Lipid Research. Our work benefited from collaboration with D.J. Patel at Memorial Sloan Kettering Cancer Center (MSKCC), L. Malinina at CIC bioGUNE (Derio/Bilbao, Spain) and J.G. Molotkovsky at the Shemyakin Institute of Bioogranic Chemistry at the Russian Academy of Science.

Recent studies have led to discovery of new GLTP superfamily members, ceramide-1-phosphate (C1P) transfer proteins (CPTPs). In Nature, we reported structural characterization of human CPTP (collaboration with D.J. Patel, MSKCC), CPTP intracellular location in mammalian cells using state-of-the-art fluorescence microscopy (collaboration with Ted Hinchcliffe, UMN-Hormel Institute), and showed that CPTP depletion in human cells by RNAi leads to C1P over-accumulation in the trans-Golgi (collaboration with Charles Chalfant, Univ. South Florida).

"Unraveling how sphingolipid transfer proteins control inflammation and programmed cell death by regulating sphingolipid levels and locations in cells is of fundamental importance for developing new therapeutic approaches to treat diseases such as cancer ."

Rhoderick "Rick" Brown



FROM LEFT TO RIGHT:
Rhoderick (Rick) Brown, Yong-guang Gao, Lucy Malinina, Shrawan Mishra (not pictured)

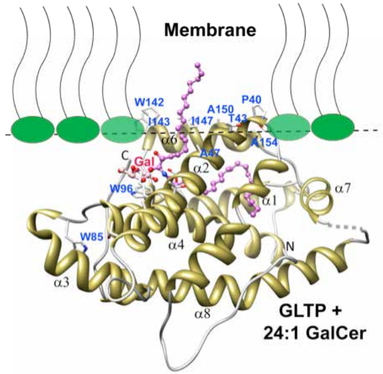
Current research projects:

1) Structural Characterization and Function of Sphingolipid Transfer Proteins in Eukaryotes
2) The Functional Role of the cPLA2α/C1P Interaction in Sepsis Resolution
3) Inflammation in Breast Cancer Initiation and Progression: Intervention Potential by Sphingolipid Transfer Proteins
4) Regulation of Ceramide-1-Phosphate Transfer Protein (CPTP): New Strategy for Enhancing Breast Cancer Therapeutics

The C1P over-accumulation triggers cytoplasmic phospholipase A2 action to generate arachidonic acid and downstream pro inflammatory eicosanoid production. Ongoing studies of human cytoplasmic phospholipase A2 (cPLA2α) are aimed at understanding how cPLA2α can initially promote but subsequently help reverse and resolve sepsis-related inflammation. We recently published new insights into the selection mechanism used by cPLA2α to target certain phospholipids in eLife. Our discoveries could help develop new treatments for sepsis and other inflammation-associated pathologic conditions, i.e. cancer, diabetes and dementia.

In studies of plant CPTP, ACD11, X ray structures published in Cell Reports in collaboration with D.J. Patel (MSKCC) and John Mundy (Univ. Copenhagen) revealed that ACD11 is a GLTP-fold evolved to bind and transfer C1P and phytoC1P. Disruption of acd11 gene impairs plant development by severely altering C1P and ceramide levels to drive programmed cell death. The findings stimulated recent studies reported in Autophagy, showing that human CPTP functions an endogenous regulator of autophagy and inflammasome assembly that drives interleukin release (IL1B and IL18).

Our studies of GLTP superfamily proteins will likely facilitate their development as nano-devices for targeted manipulation of cellular sphingolipid composition. Such strategies could help selectively destroy cancer cells and lead to new therapeutic approaches to treat other diseases. The exciting research progress emphasizes the need for continuing studies of GLTP, CPTP, and related proteins which has been fueled by financial support from NIH National Institute of General Medical Sciences, NIH National Heart Lung and Blood Institute, NIH National Cancer Institute, Southern Minnesota's Paint-the-Town Pink Initiative, and The Hormel Foundation. For more details regarding our research activities, expertise and scientific publications, please visit the following web sites:



REB Lab research activities:
https://www.hi.umn.edu/portfolio-items/rhoderick-e-brown/

ORCID ID (REB):
https://orcid.org/0000-0002-7337-3604

Experts-UMN (REB research expertise and publications):
https://experts.umn.edu/en/persons/rhoderick-e-brown

Other professional activities:

- Biophysical Society
- American Society for Biochemistry and Molecular Biology
- Membrane Structure & Assembly Subgroup, Biophysical Society
- Ad hoc NIH Study Section member and consultant
- National Science Foundation reviewer
- Japan Society for Promotion of Science reviewer
- Chemistry & Physics of Lipids, Editorial Board
- Journal of Lipids Editorial Board

Cancer Stem Cells and Necroptosis

Ilana Chefetz Menaker, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

Our lab studies the molecular and metabolic aspects of cell programmed necrosis (necroptosis) in order to design targeted therapies and prevent recurrent disease. Cell programmed necrosis or necroptosis is a recently identified novel regulated cell death pathway. Cell death with necrotic morphology and features though to be a non-regulated and uncontrollable event associated with cell injury, inflammation or ischemia. However, recent studies now reveal that necrosis can occur in regulated manner. Necroptosis participates in pathogenies of diseases including neurodegeneration, ischemia and heart disorders, and viral infections; thus targeting necroptosis will prevent or mitigate undesirable cell death. On the other hand, drugs, inducing necroptotic cell death in tumors, can potentially overcome drug resistance in cancer cells due to elevated expression of anti-apoptotic proteins. Thus, elucidation of necroptosis/ cell proliferation or necroptosis/apoptosis balance is essential to trigger cancer cell death or prevent pathological conditions such as ischemia or inflammation. The most studied kind of necroptosis is initiated by TNF-α while Caspases are inhibited using pan-Caspase inhibitor ZVAD-FMK, requires the kinase activity of Receptor-interacting proteins 1 and 3 (RIPK1 and RIPK3) followed by their downstream target MLKL.

"We are trying to identify and characterize novel targeted therapies that target specifically Cancer Stem cells taking into consideration that tumor cells are heterogeneous."

Ilana Chefetz Menaker

Current research projects:

1) Novel combination therapy in high grade serous ovarian cancer- dual inhibition of ALDH1A and MEK1/2
2) Novel compounds that target Oxidative Phosphorylation as a preferable source of energy of ovarian cancer stem cells
3) Effect of exosomes on ovarian cancer cell, purification and characterization

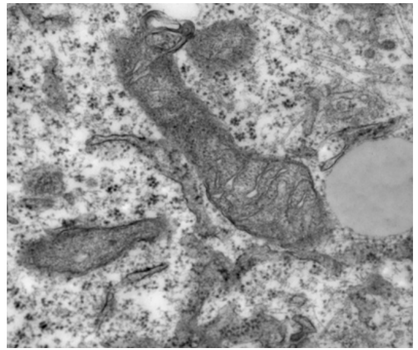


Figure 1: Transmission Electronic Microscopy of mitochondria.

FROM LEFT TO RIGHT:
Front row: Anmbreen Jamroze, Ilana Chefetz Menaker, Golla Naresh
Back row: Mary Willrodt, Mikhail Chesnokov, Imran Kha

Necroptosis execution involves formation of micro-complex (20 MDa) necroptosome followed by disintegration of mitochondrial and plasma membranes. Despite necroptosis importance, many molecular downstream events are unknown or being disputed.

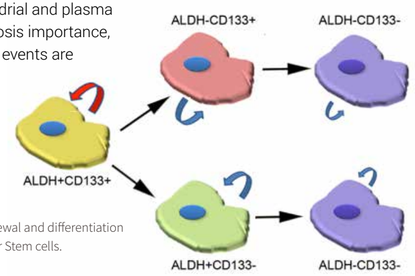


Figure 2: Self renewal and differentiation capacity of Cancer Stem cells.

Invited seminars:

1) A Novel Pan-ALDH1A Inhibitor Induces Necroptosis in Ovarian Cancer Stem-Like cells. September 21 st, 2017. Mayo clinic, Ovarian cancer spore group
2) A Novel Pan-ALDH1A Inhibitor Induces Necroptosis in Ovarian Cancer Stem-Like cells. June, 2018. University of Chicago, OBGYN department

Genome Instability & Chromosome Biology

Rafael Contreras, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

My research interests are focused on investigating the function of repetitive areas of the human genome. I have a special interest in centromere genomics. The centromere is the structural unit responsible for the correct segregation of chromosomes during cell division. Destabilization of centromere function results in chromosomal mis-segregation and instability, hallmarks of fibrosis, cancers and birth defects. I am investigating the structure and evolution of centromere sequences, the epigenetic interactions of chromatin factors that modulate centromere function on centromere sequences, and the role these elements play in chromosome segregation and genome instability in non-disjunction disorders, cancers, and fibrosis.

Human Centromere Genomics

My research has been focused on investigating Human Endogenous Retroviruses, repetitive elements that comprise 8% of the human genome. During these studies, we discovered and characterized thousands of endogenous retroviruses of the HERV-K family, in particular the types K111 and K222, which reside in the centromeres of the human genome (Contreras-Galindo et al. 2013; Zahn et al. 2015). These findings are giving us the opportunity to study human centromere genomics, regions of the human genome that have been extremely difficult to annotate because of the repetitive nature of these areas, and therefore, remain a last frontier of human genetics. Sequence analysis of centromere K111 and K222 retroviruses revealed that, surprisingly, human centromeres have been under continuous reshuffling during evolution and exchanged genetic material at rates higher than other areas of the chromosomes. We are also characterizing other centromere repetitive elements, the so-called alpha repeats, which are the main arrays of repetitive elements in human centromeres and that are found in a unique structure in each human chromosome. In the last years I have annotated centromere sequences to fill the gaps of human centromere maps. This is very important for human genomics, as recent evidence from our lab and other investigators has indicated that specific alpha repeats recruit centromeric chromatin factors that assemble the kinetochore. With the support of a Career Development Award K22 by NCI we have identified markers for multiple centromere arrays in each human chromosome and developed molecular tools to study these elements. We have studied the composition of centromeric sequences, their genetic variation in human populations and identified functional elements in the centromeres of each chromosome that modulate centromere assembly and function. We have discovered centromere markers for each human chromosome except for Chr 19. Using these markers, we have identified startling evidence of extensive centromere DNA instability in disorders characterized by chromosome mis-segregation such as trisomy 21, as well as scleroderma fibrosis and cancers. We plan to study the effect of centromere instability in centromere function and chromosome instability in these diseases.

Centromere Function and Chromosome Segregation

We are studying which centromere alphoid arrays in each human chromosome are packed into nucleosomes by the histone 3-like centromere mark CENP-A and recruit other centromere proteins (CENP-B, CENP-C, and so on) that participate in kinetochore assembly. Further, we are also characterizing which centromere sequences are essential to maintain the cohesiveness of sister chromatids during cell division through epigenetic factors HP1 and H3K9me3; factors that ensure the correct partition of chromosomes. We have developed ChIP-PCR and ChIP-Seq methods to assess this task. We have elucidated which centromere arrays in each human chromosome bind proteins that recruit kinetochores, as well as sequences in the centromere flanks that keep sister chromatids together. These novel findings are reported in a recent manuscript accepted for publication in the journal Genome Research. It is very important to understand how the deposition and assembly of centromeres as well as cohesion function is influenced when centromere sequence mutations arise. We are employing CRISPR Cas9 to mutate centromeres. We are studying whether mutations in centromere elements as we have seen in disease alter the deposition of kinetochores towards juxtaposed arrays that remain intact and/or create neo-centromeres in other areas of the chromosomes. The hypothesis is that that mutations in the centromere sequence will affect the balanced deposition of centromere chromatin marks. We anticipate that mutation of centromere elements will displace the occupancy of CENP-A, CENP-B, and/or H3K9me3 toward other elements, affecting centromere/pericentromere architecture, and therefore, chromosomal segregation. We predict increased occupancy of centromere marks on ectopic chromosomal arms with the establishment of neo-centromeres as the original centromere mutates and becomes inactive. These technologies will generate unprecedented findings in the study of human centromeres in disease.

Primary Research Areas:

1) Chromosome Biology
2) Molecular Biology
3) Genetics and Genomics

Centromeres in cancer

Using molecular tools to study human centromeres, we made made the observation that in contrast to the size of centromere elements detected in healthy cells, significantly smaller centromere arrays are found in the genome of most cancer cells studied, including breast, ovarian, and prostate cancer, lymphoma/leukemia, and teratocarcinoma. This suggests that cancer cells, which display extensive chromosome ploidy, exhibit profound centromere instability. Interestingly, the loss of centromere retroviruses K111 at the pericentromeres, specifically in adult T-cell leukemia/lymphoma (ATL) cell lines, for example, was the result of recombinational deletion among K111s. In other cancers, deletion of some centromere retroviruses resulted in selection of new mutant centromere retroviruses. Currently, I am recreating these mutations occurring in cancer cells in chromosomally normal human cells using CRISPR Cas9 and Cre-LoxP to determine the effect of centromere instability on centromere assembly and function and chromosome segregation. These studies will help us to understand the role of centromere deletion in genome instability and aneuploidy seen in cancer. We are studying the effect of centromere mutations in genome instability/aneuploidy, cell growth and proliferation, differentiation, and cell transformation. We will expand these observations in vivo using nude *in vivo* models transplanted with cells with centromere defects.

Centromere defects in Trisomy 21

We have found extraordinary evidence of centromere instability specifically in the centromere core and pericentromere of Chr 21 in individuals with trisomy 21 (Down syndrome) but not other chromosomes, suggesting that centromere 21 instability is associated with this Chr 21 non-disjunction disorder. We are creating specific deletions in the centromere of Chr 21 in karyotypically normal cells and human embryonic stem cells (hESc) using CRISPR Cas9 technology in order to characterize the effect of these genetic imbalances in centromere function and Chr 21 segregation. We are also using cytogenetic analysis, including IF-FISH and other assays for chromosome segregation to study the effect of centromere 21 mutations in trisomy 21 development and also in the development of disease-associated phenotypes seen in different cells types of patients with trisomy 21. We are creating centromere 21 mutations in iPSc that differentiate into brain cells to study the effect of centromere instability in trisomy 21 brain development and Alzheimer. Some of these findings are shown in the journal Genome Research.

Centromeres in Scleroderma

Last but not least, we have a special attention on the function of centromeres in Scleroderma as patients with this disease produce antibodies directed to centromeres. These antibodies were fundamental for the discovery of human centromeres and kinetochores. Besides the novel finding of centromere instability of specific chromosomes in Scleroderma fibroblasts, we have seen that centromere epigenetic factors are also defective in Scleroderma fibroblasts, a finding that correlate with the production of centromere antibodies in these patients. As both centromere genetics and epigenetics defects, together with an immune response to centromeres is seen in Scleroderma patients, we are clarifying whether Scleroderma is a centromere disease with very promising findings. This research is supported by the Scleroderma Foundation.

Research Specialties:

1) Pathogenesis of Scleroderma Fibrosis and Cancer
2) Genomics of Human Centromeres
3) DNA instability and Repair of Centromeres
4) Genome editing of centromeres using CRIPR Cas9 and Cre-LoxP
5) Deep-sequencing of centromeres

DNA Repair & Genome Stability

Wioletta Czaja, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

"DNA repair plays an active and critical role in cancer development and treatment. Robust and tightly regulated DNA repair systems protect our cells from cellular transformation. In many different cancer types DNA repair processes are highly dysregulated creating a unique opportunity for selective killing of cancer cells, without harming the normal cells. Our studies will reveal new mechanisms of tumor suppression and identify novel targets in personalized cancer therapy."

Wioletta Czaja

Summary

Nearly all forms of cancer contain significant levels of genomic instability (high frequency of mutations, chromosomal rearrangements), resulting from deficient or dysregulated DNA repair processes. The molecular events leading to genomic instability are not well under-stood. We investigate the fundamental mechanisms protecting genomic integrity, with a special focus on the epigenetic and chromatin-based regulation of DNA damage repair. We are also working on development of new approaches enabling direct detection, and genome-wide profiling of damaged and modified DNA bases, with the goal of uncovering novel mechanisms involved in mutagenesis and carcinogenesis. The long-term objective of our research is to understand how stability of the human genome is maintained and regulated in various cells and tissues, and to apply this new knowledge to promote advances in novel anti-cancer therapy and personalized medicine. We employ complementary approaches in biochemistry, cell and molecular biology, genetics and genomics using fungal model organisms (yeast-Saccharomyces cerevisiae) and human cell lines.

New tools in cancer risk identification and prevention; Development of new methodology ADA-SMRT enabling direct, genome-wide profiling of mutagenic alkyl DNA adducts. Alkyl DNA adducts are cytotoxic and mutagenic DNA lesions that arise from exposure of cells to numerous environmental carcinogens and cellular metabolites. Some of the most toxic alkyl DNA adducts are induced by commonly used anti-cancer drugs. DNA modifications induced by alkylating agents play significant roles in both the development and treatment of cancer.

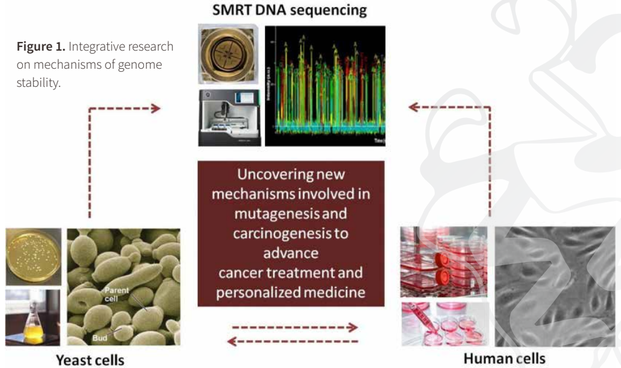


Figure 1. Integrative research on mechanisms of genome stability.

Persistent and inefficiently repaired alkyl DNA lesions can induce G→A transition mutations such as those often found in genes critical for malignant transformation, such as H-ras oncogene or TP53 tumor suppressor gene. DNA adducts induced by common alkylating agents predispose cells to malignant transformation. To understand how alkyl DNA adducts contribute to mutagenesis, cancer development and treatment, it is imperative to be able to examine complete, genome-wide profiles of these lesions and investigate how factors (such as modifications of chemotherapy agents, or the genomic and epigenomic landscape of the cell) influence initial adduct formation and repair. In collaboration with the University of Georgia, Athens, GA and Icahn School of Medicine, Mt. Sinai, New York, we are working on development of ADA-SMRT, a new methodology enabling direct detection of carcinogenic alkyl DNA adducts (ADA) by using SMRT DNA sequencing. The development of ADA-SMRT sequencing will enable, for the first time, direct and simultaneous high throughput mapping of various alkyl DNA adducts in eukaryotic genomes. This research will provide a framework for future investigation of the human genome and better understanding of the individual differences in cancer predisposition and response to chemotherapy.

Primary Research Areas:

1) DNA damage and repair
2) Chromatin remodeling
3) Alkylating agents
4) Carcinogens
5) Mutagenesis
6) Genome technologies

Identification of new mechanisms of tumor suppression; Epigenetic (chromatin-based) regulation of DNA repair. DNA repair capacity vary considerably between individuals and between different tissues, highlighting involvement of genetic and epigenetic (chromatin-based) mechanisms in modulation of cellular toxicity to genotoxic agents. One of the major challenges in the DNA repair field is to understand how efficient DNA repair is accomplished in the context of highly compacted chromatin, which is inherently inhibitory to DNA repair processes. Our research in this area focuses on investigating the role of the chromatin remodeling factors in modulating the repair of the alkylated DNA damage via Base Excision Repair (BER). The Base Excision Repair (BER) is considered a fundamental tumor suppressor pathway in all cells, and is an attractive target in novel anti-cancer drug discovery. However, unlike other DNA repair pathways, the chromatin-based regulation of BER has been substantially understudied. In human cells, robust and tightly regulated BER is essential for the efficient repair of alkyl DNA adducts and protection of cells from the accumulation of mutations and malignant transformation. Dysregulation of the BER pathway (BER imbalance) is thought to drive carcinogenesis and contribute to chemoresistance. Previously we identified a link between the essential SWI/SNF (SWItch/Sucrose Non-Fermentable) chromatin remodeler and the BER of alkyl DNA adducts in yeast cells. Currently we investigate the BER in human cancer cells deficient in the SWI/SNF. The human SWI/SNF remodeler is frequently mutated in 20% of cancers, raising the possibility that loss of SWI/SNF function might lead to aberrant, imbalanced BER in SWI/SNF-deficient cancers.

These studies will elucidate new chromatin-based mechanisms that modulate the responses of the human cells to chemotherapy alkylating agents, and will provide new insights for improved therapy of SWI/SNF-deficient cancers.

Funding:
R21 NIH NIEHS 1R21ES02854901A1
(PI: Czaja W)

Current projects:

1) **Role of chromatin remodeler LSH/HELLS in genome stability and cancer**
   - Investigating new role of LSH/HELLS in repair of heterochromatin domains
2) **Chromatin-based regulation of Base Excision Repair (BER)**
   - BER balance in cancer prevention and treatment
   - New link between BER and SWI/SNF tumor suppressor
3) **Development of new methodology ADA-SMRT**
   - Genome-wide profiling of the chemotherapy-induced DNA adducts

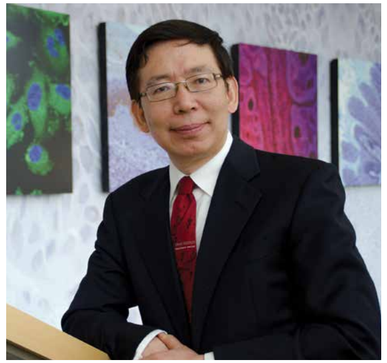
Cellular and Molecular Biology

Zigang Dong, M.D., Dr. P.H.

SECTION LEADER

MCKNIGHT PRESIDENTIAL PROFESSOR IN CANCER PREVENTION

HORMEL-KNOWLTON PROFESSOR



Summary

Cancer is one of the leading causes of human death worldwide. By focusing on its molecular mechanisms, we continue to discover key events in cancer development, as well as agents for cancer prevention and therapy.

1. Discovery of key molecular events in cancer development and metastasis

Metastasis is the major cause of death for patients with solid malignancies. We demonstrated that p53-related protein kinase (PRPK) is phosphorylated by T-LAK cell-originated protein kinase (TOPK) and promotes colon cancer metastasis. Higher expression levels of p-PRPK are associated with late stages of metastasis, stages III and IV, compared with stages I and II. We identified fusidic acid sodium salt (FA) as an inhibitor of PRPK, and showed that FA combined with 5-fluorouracil (5-FU) inhibited colon cancer metastasis to the lung.

The phosphoinositide 3-kinase (PI3-K)/Akt signaling pathway is important in the regulation of proliferation and is negatively regulated by the dual-specificity phosphatase and tensin homolog (PTEN) protein. Studies showed that PTEN phosphatase activity is inhibited by PREX2 and revealed that CELF2, an RNA binding protein, cooperates with PTEN as a tumor suppressor in cancer. CELF2 interacts with PREX2 and reduces its association with PTEN. PTEN phosphatase activity is upregulated with CELF2. CELF2 gene delivery could significantly inhibit PDX tumor growth.

2. Discovery of novel targets and agents for cancer prevention

We reported that PRPK is a novel oncogenic protein that is phosphorylated by TOPK. Knockdown of TOPK inhibited PRPK phosphorylation and conferred resistance to solar-simulated light (SSL)-induced skin carcinogenesis. We identified two PRPK inhibitors, FDA-approved rocuronium bromide (Zemuron®) or betamethasone 17-valerate (Betaderm®) and topical application of either compound decreased SSL-induced cutaneous squamous cell carcinoma (cSCC) development.

The leukotriene A4 hydrolase (LTA4H) pathway plays a role in colorectal cancer (CRC). High expression of LTA4H also associated with CRC survival probability. Samples from 13 CRC patients showed a significant decrease in the LTA4H signaling pathway in a drug (bestatin)-treated group compared with the untreated group. Bestatin effectively inhibited tumorigenesis in *in vivo* models. These results demonstrate evaluation of bestatin efficacy in CRC and support further studies focusing on LTA4H inhibition in CRC.

We report that tumor necrosis factor receptor (TNFR)-associated factor 1 (TRAF1) is overexpressed in human lung cancer cells and tissues and expression level was inversely correlated with patient survival probability. Overall, our work suggests that TRAF1 offers a candidate molecular target for lung cancer prevention and therapy.

"Cancer is one of the leading causes of human death worldwide. By focusing on its molecular mechanisms, we continue to discover key molecular events in cancer development, as well as agents for cancer prevention and therapy."

Zigang Dong

3. Discovery of novel targets and agents for cancer therapy

Esophageal cancer (EC) is one of the most aggressive malignancies of the upper aerodigestive tract. Aurora kinases play a crucial role in mitosis and we identified a novel compound, referred to as APIO-EE-9, which inhibits growth and induces death of esophageal cancer cells by interacting with both Aurora A and B. Importantly, APIO-EE-9 significantly decreased the size of esophageal patient-derived xenograft tumors suggesting that APIO-EE-9 could be developed as a therapeutic agent against esophageal cancer.

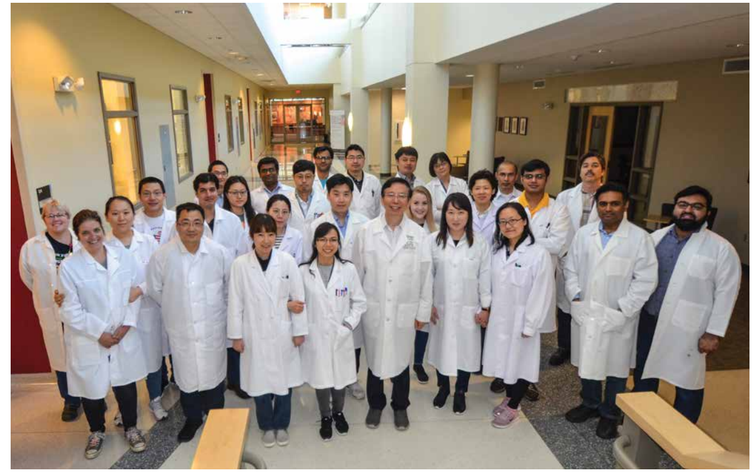
Osteosarcoma is the primary human malignant tumor affecting bone and prognosis is very poor. Aurora B kinase is overexpressed in osteosarcoma compared with normal bone tissue. We developed a compound, HOI-07, as a specific Aurora B kinase. Results of a xenograft *in vivo* model study indicated that HOI-07 treatment suppressed the growth of osteosarcoma cell growth.

Purpurogallin is a natural compound that suppresses esophageal cancer cell (ESCC) growth by directly targeting the mitogen-activated protein kinase kinase 1/2 (MEK1/2) and suppressing the extracellular signal-regulated kinase 1/2 (ERK1/2) signaling pathways to inhibit growth.

Oridonin, a natural compound, is an inhibitor of AKT and suppresses proliferation of ESCC by blocking AKT kinase activity and inhibited growth of esophageal cancer cells. A combination of oridonin and 5-FU or cisplatin enhanced the inhibition of ESCC cell growth. The effects of oridonin were verified in patient-derived xenograft tumors expressing high levels of AKT.

Gossypin is a flavone, that is an Aurora kinase A (AURKA) and RSK2 inhibitor that suppresses gastric cancer growth by directly binding and inhibiting AURKA and RSK2 activities and their downstream signaling proteins.

Malignant melanoma is an aggressive tumor of the skin that lacks effective preventive and therapeutic treatments. In melanoma, both the BRAF/MEK/ERK and PI3-K/AKT signaling pathways are overactivated, resulting in uncontrolled growth. We found that Ashitaba chalcones, 4-hydroxyderricin (4HD) and xanthoangelol (XAG), suppressed melanoma development by directly targeting both BRAFV600E and PI3-K leading to the death of melanoma cells and thus are promising chemopreventive or therapeutic agents against melanoma.



FROM LEFT TO RIGHT:
First Row: Tara Adams, Hanyong Chen, Hisae Yoshitomi, Thi My Le Le, Zigang Dong, Eunmiri Roh, Qiushi Wang, Kanamata Reddy, Muhammad Azeem Saeed
Second Row: Teri Johnson, Xinli Ma, Jian Li, Faisal Aziz, Yifei Xie, Seung Ho Shin, Tressie Kinney, Ke Yao, Abhijit Chakraborty, Humberto de Vitto
Third Row: Junsheng Zhu, Jinhua Wang, Souren Paul, Joohyun Ryu, Moeez Rathore, Xiaoyu Chang, Tianshun Zhang, Wei-Ya Ma, Raja Dey

Cell Death and Cancer Genetics

Yibin Deng, M.D., Ph.D.

SECTION LEADER / I.J.HOLTON PROFESSOR

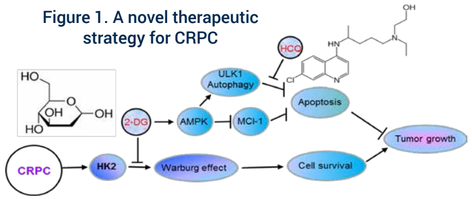
Summary

My research lab has been utilizing genetic engineered *in vivo* models to address whether and how the genetic changes identified in cancer patients contribute to tumor initiation, progression and metastasis *in vivo*; utilizing multiple approaches including genome editing technology (CRISPR-Cas9), X-ray crystallography and Cryo-EM to functionally and structurally understand how oncogenes are selectively translated *in situ* in tumorigenesis; and utilizing computational drug target screen and design to discover novel small molecular compounds that selectively target human cancer cells while sparing normal cells. Accordingly, we have established a strong collaborative team that brings together expertise from the areas of cancer biology, medicinal chemistry, structural biology, pathology, preclinical genetic *in vivo* models, and clinical practice aiming to identify the Achilles' heel of cancers that can serve as a means to selectively and effectively kill cancer cells.

1. Discovering a gene-regulated metabolic target for currently incurable castration-resistant prostate cancer

Prostate cancer strikes one in six men and is the second leading cause of cancer-related deaths in men after lung cancer in the United States. Androgen deprivation therapy (ADT) by surgical and/or chemical castration remains the gold standard-of-care therapy for metastatic prostate cancer. Unfortunately, these prostate cancers invariably develop resistance to ADT and progress to a more aggressive castration-resistant prostate cancer (CRPC). Currently, no therapeutic options exist for CRPC patients who have developed resistance to the second generation of anti-androgen receptor (AR) signaling axis therapy.

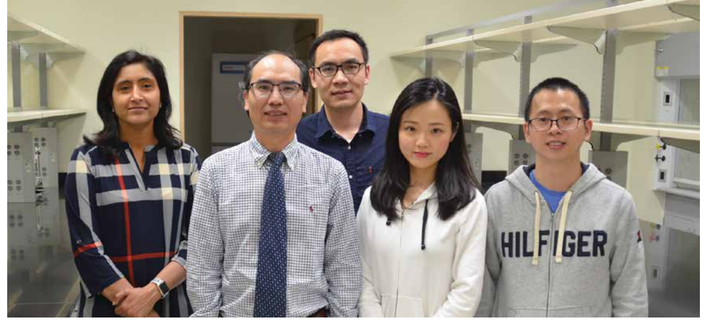
Figure 1. A novel therapeutic strategy for CRPC



Clinically, approximately 30% of CRPC patients carrying both *PTEN* mutation and AR amplification while additional 30% of CRPC patients harboring co-deletion or -mutation of *PTEN* and *TP53*. Our latest data show that HK2 (hexokinase 2) protein, which catalyzes the irreversible first step of glycolysis by phosphorylating glucose to glucose-6-phosphate, is selectively induced in CRPC harboring *PTEN* loss/p53 mutation or *AR* amplification; Mechanistically, we demonstrate that (a) *AR* amplification increases hexokinase 2 (HK2) mRNA transcription through direct occupation at the AR binding sites (ARBS) within HK2 gene; (b) p53 deficiency increases HK2 mRNA through inhibition of miR143-mediated HK2 mRNA degradation; and (c) *PTEN* loss selectively enhances HK2 mRNA translation (protein synthesis) through AKT1-mTORC1-eIF4A1-mediated axis; Genetically, we demonstrate that HK2-mediated Warburg effect is required for CRPC tumor growth *in vivo*; Pharmacologically, we demonstrate that co-targeting the HK2-mediated Warburg effect with 2-deoxyglucose (2-DG, a hexokinase inhibitor) and ULK-1-dependent autophagy with FDA-approved chloroquine (CQ) or less toxic quinolone-derivative hydroxychloroquine (HCQ) efficiently kills HK2-dependent CRPC cells and remarkably extends host survival in CRPC *in vivo* models (**Figure 1**). Taken together, our recent findings strongly support that targeting HK2-mediated Warburg effect as a selective and effective strategy for currently incurable CRPC.

"Our studies will reveal whether and how the genetic changes identified in cancer patients lead to tumorigenesis in vivo and identify effective strategies targeting cancerous cells while leaving normal cells untouched."

Yibin Deng

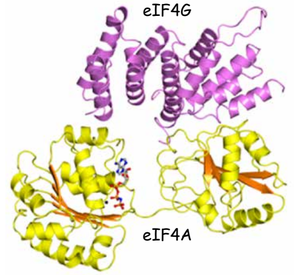


FROM LEFT TO RIGHT:
Puja Singh, Yibin Deng, Zhengbo Song, Yu Wang, Peng Li

2. Understanding oncogene mRNA translation in tumorigenesis

Dysregulated messenger RNA (mRNA) translation into oncogene protein plays a crucial role in tumorigenesis. mRNA translation regulation occurs at multiple steps of the translation process, but it is largely achieved during translation initiation, which is predominantly mediated by the eukaryotic translation initiation factor 4F (eIF4F) heterotrimeric complex composed of three subunits eIF4E, eIF4A and eIF4G. Biochemical studies suggested that interaction of eIF4A and eIF4G plays a crucial role in initiating oncogene mRNA translation in cancer cells.

Figure 2. Crystal structure of human eIF4A/eIF4G complex



We have been utilizing x-ray crystallography to successfully solve the complex structure of human eIF4A/eIF4G (**Figure 2**). Site-mutagenesis studies reveal the crucial residues contributing to the complex formation and tumorigenesis *in vivo*. Our studies thus provide a structure-based discovery of small-molecule compounds that disrupt the eIF4A/eIF4G interaction in cancer cells to selectively block oncogene protein synthesis and inhibit tumor development *in vivo*. Currently, we are combining X-ray crystallography, Cryo-electron microscopy (cryo-EM), and biological studies to decipher how the eukaryotic translation initiation complex involved in selective oncogene mRNA translation, which leads to tumorigenesis.

3. Dissecting the role of dysfunctional telomere-induced DDR-STING innate immune response in tumorigenesis

Telomeres are nucleoprotein caps that protect chromosomal ends from being recognized as aberrant damaged DNA and prevent chromosome end-to-end fusions. Dysfunctional telomeres could arise either from progressive telomere attrition (telomere shortening) or when components of the telomeric DNA-binding proteins – termed "shelterin complex" – are perturbed (telomere uncapping). Our laboratory has been engineering a novel breast and prostate cancer models harboring telomere uncapping, which in turn induces DNA damage response **(DDR)-STING/TBK1-IRF3** innate immune pathway *in vivo*. We have been utilizing these novel breast/prostate cancer models to dissect the role of immune response in telomere dysfunction-driven tumorigenesis *in vivo* and stimulate the immune pathways to effectively kill human breast/prostate cancer cells.

Our research projects are well supported by the grants from The University of Minnesota, Hormel Foundation and National Cancer Institute of NIH (R21s and R01s).

Other professional activities:

Standing Member, National Cancer Institute Study Section; Reviewer for Scientific Journals

Cancer Cell Biology and Translational Research

Sergio Gradilone, Ph.D.

SECTION LEADER / ASSOCIATE PROFESSOR

Summary

The "Cancer Cell Biology and Translational Research" section focuses on understanding the basic biological processes involved with a normal cell transforming into a cancerous one. By understanding these mechanisms, potential therapeutic interventions may be envisioned. We continue investigating the role of the primary cilium in tumor biology. Primary cilia are multisensory organelles – similar to a cell antenna – that sense and receive signals from the environment surrounding the cells. We've found that these antennae are lost in tumor cells; therefore, we are trying to understand the mechanisms of ciliary loss, and what are the consequences of such a loss. Furthermore, as we gain knowledge on these mechanisms, we are now able to induce the restoration of primary cilia in tumor cells and bring back the malignant cells to a more normal phenotype, which may contribute to the development of new therapeutic strategies based on the rescue of primary cilia integrity.

1 - Mechanisms of Ciliary Loss

Project 1

Cholangiocarcinoma (CCA) is a lethal malignancy with features of biliary tract differentiation. Importantly, more than 2,500 new cases of CCA are diagnosed in the USA each year, and, in the last 4 decades, incidence rates of intrahepatic CCA have increased by 165%. Therefore, it is imperative to identify the cellular networks regulating disease initiation and progression to develop better therapies. Thus, our long-term goals are to understand the pathogenesis of CCA and develop new therapies for its treatment. Our current overall objective is to explore the importance of cholangiocyte deciliation, a pathologic process recently discovered by us in the pathogenesis and progression of CCA. We propose here that autophagy, a physiological process that degrades organelles, and ubiquitination, a process involved in protein degradation, are linked to ciliary disassembly by a mechanism dependent on HDAC6, which is a protein overexpressed in tumor cells and induces the resorption of cilia by regulating ciliary stability and the transport of its components to the cellular degradative compartments (**Figure 1**). We hypothesized that by pharmacological inhibition of HDAC6 and/or autophagy, it is possible to restore the expression of cilia on tumor cells and transform them back to a less aggressive and more normal phenotype. The autophagy inhibitor hydroxychloroquine has been extensively used in humans as an antimalarial treatment, therefore it is readily available for use in our research. Our experiments are evaluating the effects of combined HDAC6 and autophagy inhibition *in vitro* and *in vivo*, laying the foundation for a potential clinical trial.

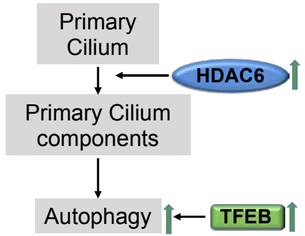


Figure 1. ***Ciliophagy model.*** The orchestrated work of over-expressed HDAC6 and increased autophagy in CCA induces the resorption of cilia and the degradation of the ciliary components by autophagy.

"Our research is uncovering novel and generalizable information on fundamental, ciliary-dependent mechanisms controlling the proliferation and migration of malignant cells, and provide the foundation for anti-cancer therapies based on the rescue of primary cilia functions, i.e Ciliotherapy."

Sergio Gradilone

2 - Consequences of Ciliary Lost

Project 2

Primary cilia that can detect several kinds of environmental signals and then transmit this information into the cell. Here, we tested the hypothesis that the chemosensory function of cholangiocyte primary cilia acts as a mechanism for tumor suppression. We found that in the presence of extracellular nucleotides, ciliary-dependent chemosensation of the nucleotides inhibited migration and invasion in normal ciliated cholangiocytes via a P2Y11 receptor and LKB1-PTEN-AKT dependent mechanism. In contrast, in normal experimentally deciliated cholangiocytes and tumor cells, the nucleotides induced the opposite effects, i.e. increased migration and invasion. As activation of LKB1 via a ciliary dependent mechanism was required for the nucleotide-mediated inhibitory effects on migration and invasion, we attempted to activate directly LKB1, independent of ciliary expression, using the compound hesperidin methyl chalcone (HMC). We found that HMC induced activation of LKB1 in both ciliated and deciliated cells in vitro, resulting in the inhibition of migration and proliferation. Furthermore, using an *in vivo* CCA model, we found that HMC inhibited tumor growth. These findings highlight the importance of the chemosensory function of primary cilia for the control of migration and invasion, and suggest that by directly activating LKB1 and bypassing the need for primary cilia, it is possible to emulate this chemosensory function in CCA cells (**Figure 2**). These data warrant further studies for evaluating the possibility of using HMC as a novel therapy for CCA.

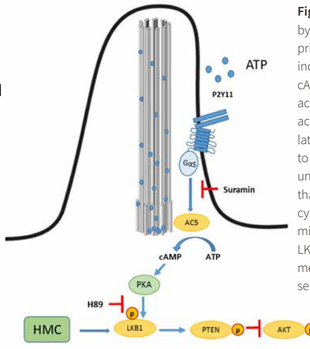


Figure 2. Nucleotides are detected by P2Y11 receptor localized in the primary cilium activating AC5, which induces increases in the levels of cAMP and activation of PKA. Once activated PKA phosphorylates and activates LKB1, LKB1 phosphorylates and stabilizes PTEN, leading to AKT inhibition. Inhibited AKT is unable to phosphorylate F-actin that is required to reorganize the cytoplasm and protrusions in migrating cells. HMC can activate LKB1 via a ciliary-independent mechanism emulating the chemosensory function of primary cilia.

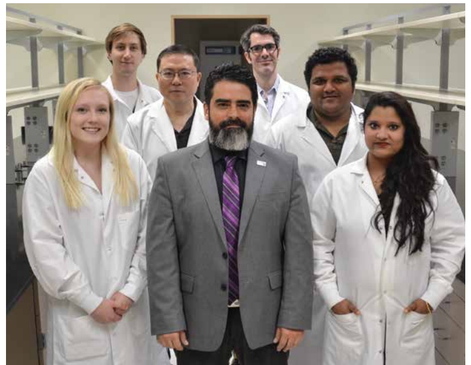
3 - New therapies and translational studies

Project 3

In collaboration with Dr. George Alslanidi, we aimed to develop safe and effective vectors based on a human virus, the adeno-associated virus (AAV), for genetic manipulation of bile duct cells. Our proposed studies are designed to develop effective vectors through the manipulation of the viral capsid structures and expression cassettes for specifically targeting CCA tumor cells. Our overall objective is to develop new gene therapy tools for specifically targeting CCA tumor cells and also manipulate the expression of important target-genes in these cells that were recently identified by us and could help devise novel therapies. This virus causes no known disease and currently being used in a number of clinical trials for the potential treatment of a wide variety of human diseases.

Project 4

Other studies in the Section in collaboration with Dr. Da-Qing Yang include the investigation of two important proteins, p53 and ATM, which are critical for multiple physiological processes, including cell cycle progression, DNA damage repair, insulin signal transduction, and glucose metabolism. The study of the translational regulation of p53 induction following DNA damage provides better understanding regarding how defective synthesis of the p53 tumor suppressor is involved in the development of cancer, which may lead to novel diagnosis and treatment strategies for various types of cancer, including breast, prostate, and pediatric cancer. A collaborative project between Dr. Yang and Dr. Gradilone is to restore p53 tumor suppressor function in breast cancer cells by inhibiting the HDAC6 activity. Signal transduction of the ATM protein kinase in response to insulin and metformin is known to be linked to both cancer and diabetes. Inhibition of ATM-mediated glucose uptake and aerobic glycolysis has shown great potential in preventing mammary tumor development and in suppressing cancer cell survival.



FROM LEFT TO RIGHT:
Front Row: Trinity Grey, Sergio Gradilone, Aalekhya Biswas
Back Row: Seth Richard, DaQing Yang, Estanislao Peixoto, Kishor Pant

Cellular Dynamics

Edward H. Hinchcliffe, Ph.D.

SECTION LEADER / PROFESSOR

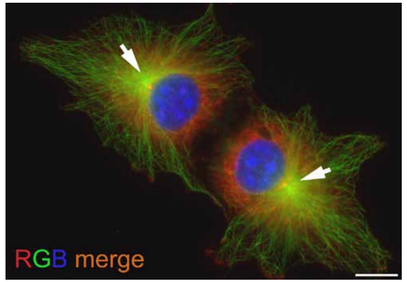
"We focus on the role of mitotic mechanisms in the generation of human disease."
Edward H. Hinchcliffe

Summary

Our work seeks to understand the cellular basis for tumorigenesis. We study the process where cells separate duplicated chromosomes into two daughter cells - called cell division or mitosis. Mistakes in mitosis lead to uneven chromosome segregations, which is a hallmark of cancer progression. Specifically, we study how changes to DNA (mutations) lead to chromosome missegregation during mitosis. By understanding the molecular mechanisms underlying these cellular defects, we will provide insight into new methods of diagnosis, prevention and treatment for cancer.

Chromosome missegregation: Inadvertent chromosome missegregation in anaphase generates aneuploid cells, but the proliferation of these cells is normally blocked, because chromosome missegregation also triggers a p53-dependant failsafe that triggers cell cycle arrest in the ensuing G1. The molecular mechanisms underlying this trigger are not known.

Recently we have identified a conserved feedback mechanism that monitors the relative position of lagging chromosomes during anaphase via the differential phosphorylation of the histone variant H3.3 at Ser31. During normal mitosis H3.3 Ser31 is phosphorylated exclusively at peri-centromeres, which are rapidly dephosphorylated in anaphase. We induced non-transformed cells to missegregate chromosomes by transiently depolymerizing spindle microtubules with cold. These cells undergo the metaphase-anaphase transition in the presence of one or more misaligned chromosomes that lack BubR1 labeling. These cells transit mitosis with relatively normal timing and lack DNA damage. After re-warming, correlative same cell live and fixed imaging revealed that isolated chromosomes (e.g. lagging in anaphase) have hyper-phosphorylated H3.3 Ser31 (pS31) along their arms that persists into G1 as these chromosomes assemble into a micronucleus. Surprisingly, during telophase Ser31 phosphorylation along individual chromosomes initiates global phosphorylation of H3.3 Ser31 in both reforming nuclei, suggesting both an amplification step of the aneuploid failsafe, and an explanation for why both daughter cells trigger p53 activation in response to a single chromosome missegregation event. pS31 is mimicked by the hyperlocalization of ATRX to isolated chromosome arms. ATRX – a member of the SWI/SNF family of chromatin binding protein – is known to load histone H3.3 into nucleosomes. Unlike H3.3 S31 phosphorylation during anaphase, the association of ATRX with isolated chromosomes is transient; by nuclear envelope reformation ATRX is absent from the resulting micronucleus.

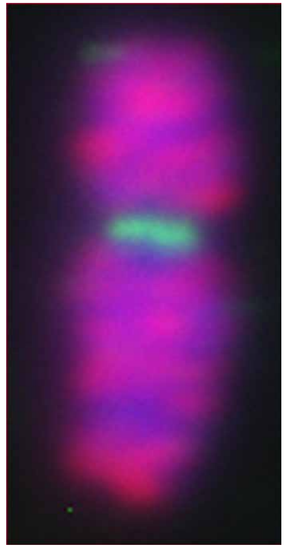


Cultured cells showing position of centrosome (arrows)

Finally, we demonstrate that post-anaphase H3.3 pS31 and ATRX are required to trigger p53 stabilization in the subsequent G1. Microinjection of monospecific antibodies against either pS31 or ATRX into anaphase cells containing lagging chromosomes blocks p53 accumulation in G1 nuclei¬. Here we show that p53 cell cycle arrest – triggered by chromosome missegregation – is mediated via a novel signaling mechanism dependent upon H3.3 S31 phosphorylation and ATRX recruitment to lagging chromosomes. This work provides insight into how aneuploidy is normally monitored and suppressed. Furthermore, driver mutations in H3.3 (flanking Ser31) and null mutations in ATRX are both found in pediatric glioblastomas, suggesting that disrupting the aneuploidy failsafe contributes to neoplastic progression.

FROM LEFT TO RIGHT:
Alyssa Langfald, Charles Day, Edward Hinchcliffe, Paiton Schwab

The role of the centrosome in mitotic spindle assembly: The role of centrosomes/centrioles during mitotic spindle assembly in vertebrates remains controversial. In cell-free extracts and experimentally derived acentrosomal cells, randomly oriented microtubules (MTs) self-organize around mitotic chromosomes and assemble anastral spindles. However, vertebrate somatic cells normally assemble a connected pair of polarized, astral MT arrays – termed an amphiaster ("a star on both sides") – that is formed by the splitting and separation of the microtubule-organizing center (MTOC) well before nuclear envelope breakdown (NEB). Whether amphiaster formation requires splitting of duplicated centrosomes is not known. We found that when centrosomes were removed from living vertebrate cells early in their cell cycle, an acentriolar MTOC re-assembled, and prior to NEB, a functional amphiastral spindle formed. Cytoplasmic dynein, dynactin, and pericentrin are all recruited to the interphase aMTOC, and the activity of kinesin-5 is needed for amphiaster formation. Mitosis proceeded on time and these karyoplasts divided in two. However, ~35% of aMTOCs failed to split/separate before NEB, and these entered mitosis with persistent monastral spindles. The chromatin-mediated RAN-GTP pathway could not restore bipolarity to monastral spindles, and these cells exited mitosis as single daughters. Our data reveal the novel finding that MTOC separation and amphiaster formation does not absolutely require the centrosome, but in its absence, the fidelity of bipolar spindle assembly is highly compromised.



Isolated chromosome labeled with anti- modified histone H3.3 (red), anti-CENP A (green), and DAPI (blue)

Cancer Biology

Luke Hoeppner, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

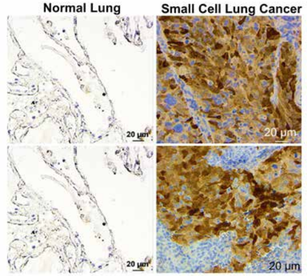
Discovering new ways to combat lung cancer is a primary focus of our "Cancer Biology" section. Our research program integrates molecular, genetic, and biochemical approaches to analyze *in vivo* models, clinical samples, and patient databases to discover novel mechanisms that regulate cancers without clear therapeutic options. Our goal is to rapidly identify potential prognostic indicators, therapeutic targets, and strategies to overcome drug resistance in lung cancer patients. Our laboratory also studies molecular mechanisms and signal transduction pathways involved in vascular permeability, angiogenesis, cancer progression, metastasis, and adverse effects of cancer therapy.

Our talented research team consists of Sk. Kayum Alam, Ph.D. and Li Wang, M.D., Ph.D., two outstanding postdoctoral research fellows. Dr. Alam is primarily focused on accelerating our lung cancer discoveries. Dr. Wang specializes in vascular biology and angiogenesis research, which is applicable to finding new treatments for cardiovascular disease and stroke as well as cancer. Our research group is pleased to be joined by Zhu Zhu, a visiting scientist who is currently learning a variety of molecular biology techniques and making important contributions to multiple research projects. Together, our group has made excellent progress on numerous research directions this past year. Highlights include discovering that DARPP-32 isoforms promote non-small cell lung cancer growth through IKKα-dependent cell migration (*Communications Biology*, 2018), showing for the first time that ASCL1-regulated DARPP-32 and t-DARPP stimulate small cell lung cancer growth and neuroendocrine tumor cell survival (preprint: *BioRxiv*, 2019; under peer review: Oncogene), contributing to work showing BMI1 is a potential therapeutic target for treatment of metastatic prostate cancer (*Clinical Cancer Research*, 2018), and collaborating with Hormel Institute colleague Dr. Rendong Yang on his development of ScanNeo, a bioinformatics tool used to identify new antigens using RNA-Seq data (*Bioinformatics*, 2019).

1. Triggering the dopamine pathway to inhibit non-small cell lung cancer progression

Lung cancer is the leading cause of cancer-related death worldwide. We demonstrate that elevated expression of dopamine and cyclic adenosine monophosphate-regulated phosphoprotein, Mr 32000 (DARPP-32) and its truncated splice variant t-DARPP promote lung tumor growth, while abrogation of DARPP-32 expression in human non-small cell lung cancer (NSCLC) cells reduces tumor growth in orthotopic in vivo models. We observe a novel physical interaction between DARPP-32 and inhibitory kappa B kinase-α (IKKα) that promotes NSCLC cell migration through non-canonical nuclear factor kappa-light-chain-enhancer of activated B cells 2 (NF-κB2) signaling. Bioinformatics analysis of 513 lung adenocarcinoma patients reveals elevated t-DARPP isoform expression is associated with poor overall survival. Histopathological investigation of 62 human lung adenocarcinoma tissues also shows that t-DARPP expression is elevated with increasing tumor (T) stage. Our data suggest that DARPP-32 isoforms serve as a negative prognostic marker associated with increasing stages of NSCLC and may represent a novel therapeutic target. We are now beginning to investigate the role of DARPP-32 isoforms in different subtypes of lung cancer, specifically EGFR mutant NSCLC and small cell lung cancer. A current area of focus is to understand how DARPP-32 and t-DARPP promote resistance to molecular targeted therapies.

"Our research team is focused on discovering the molecular basis for new and improved lung cancer therapies."
Luke Hoeppner

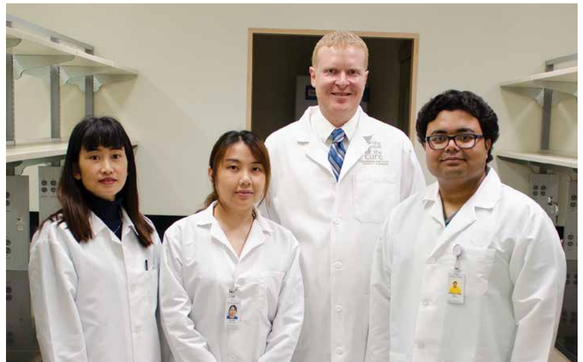


DARPP-32 proteins (stained brown) are overexpressed in human small cell lung cancer tissue, but virtually undetectable in physiologically normal human lung.

2. DARPP-32 drives small cell lung cancer growth and neuroendocrine tumor cell survival

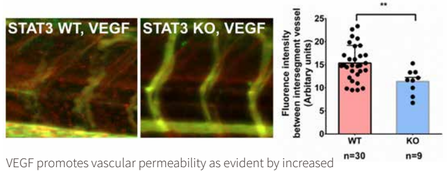
Small cell lung cancer (SCLC) is the most aggressive form of lung cancer, and new molecular insights are necessary for prognostic and therapeutic advances. We demonstrate in orthotopic models that DARPP-32 and its splice variant t-DARPP promote SCLC growth through increased proliferation, Akt/Erk-mediated survival and anti-apoptotic signaling. DARPP-32 and t-DARPP proteins are overexpressed in SCLC patient-derived tumor tissue, but virtually undetectable in physiologically normal lung. RNA sequencing analysis reveals a subset of SCLC patients with high tumoral t-DARPP expression and upregulated Notch signaling genes, including achaete-scute homologue 1 (ASCL1). We show that DARPP-32 isoforms are transcriptionally activated by ASCL1 in human SCLC cells. Taken together, we demonstrate new regulatory mechanisms of SCLC oncogenesis that suggest DARPP-32 isoforms may represent a negative prognostic indicator for SCLC and serve as a potential target for the development of new therapies.

FROM LEFT TO RIGHT:
Li Wang, Zhu Zhu, Luke Hoeppner, Sk. Kayum Alam



3. VEGF activates STAT3 to promote vascular permeability

Vascular endothelial growth factor (VEGF) induces vascular permeability in ischemic diseases and cancer, leading to many pathophysiological consequences. For example, in heart attack or stroke, expression of VEGF leads to vascular permeability, edema, and tissue damage. However, following cardiovascular or cerebrovascular infarct, VEGF promotes repair of ischemic tissue. Consequently, understanding the molecular mechanisms of VEGF-induced vascular permeability will facilitate the development of promising therapies that achieve the delicate balance of inhibiting vascular permeability while preserving ischemic tissue repair mediated by VEGF signaling. VEGF signals through its receptor, VEGFR-2, to activate signal transducer and activator of transcription 3 (STAT3). Phosphorylation of STAT3 promotes its nuclear translocation, enabling STAT3 to transcriptionally activate target genes. We show in human umbilical vein endothelial cells (HUVEC) that VEGF induces VEGFR-2/STAT3 association, STAT3 phosphorylation, and STAT3 nuclear localization. We demonstrate that STAT3 positively regulates VEGF-mediated vascular permeability by utilizing a VEGF-inducible zebrafish model in conjugation with STAT3 knockout zebrafish generated using CRISPR-Cas9 genome editing. We also confirm STAT3 promotes VEGF-induced vascular permeability in a mammalian model system. Taken together, our results suggest STAT3 plays a critical role in VEGF-induced vascular permeability, which may translate to improved therapies for patients afflicted by heart disease, stroke, or cancer.



VEGF promotes vascular permeability as evident by increased exit of red dextran from green labeled zebrafish veins (left). STAT3 knockout (KO) reduces VEGF-induced permeability (middle). Quantification of permeability in STAT3 wildtype (WT) vs. KO zebrafish (right).

Other professional activities:

Funding:
American Cancer Society
Institutional Research Grant

Editorial Board:
Nature Partner Journals: Precision Oncology

Grant Review:
National Institutes of Health
National Cancer Institute

Tumor Microenvironment and Metastasis

Ningling Kang, Ph.D.

SECTION LEADER / ASSOCIATE PROFESSOR

Summary

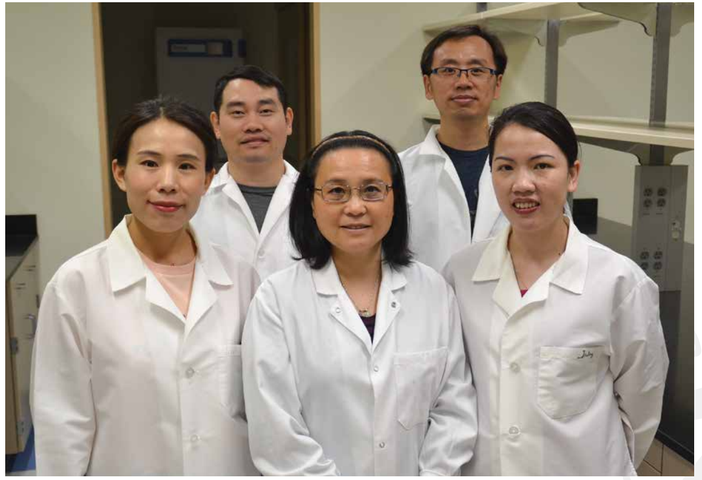
Tumor-derived factor TGFβ is the most potent cytokine that induces HSC activation. So we focus on (1) intracellular trafficking of TGFβ receptors in HSCs, (2) mechanisms by which SMAD2/3 enter the nucleus in response to TGFβ stimulation, and (3) epigenetic mechanisms and regulation of HSC activation. In addition, we also investigate the role of extracellular matrix-mediated forces in HSC activation.

In the annual report of 2018, we described that knockdown of the transcription coactivator p300 or p300 inhibitor C646 inhibited TGFβ1-stimulated nuclear accumulation of SMAD2/3 and TAZ. Additionally, deleting the nuclear localization signal of p300 (PSAKRPK) abolished SMAD2/3 and TAZ nuclear transport induced by TGFβ1. As interrogated with additional mechanistic studies in this year, we further demonstrated that p300 in fact played both non-canonical and canonical roles for HSC activation by functioning as a cytoplasm-to-nucleus shuttle for SMAD2/3 and TAZ and by epigenetically promoting gene transcription via histone acetylation and chromatin remodeling. Thus, p300 is an attractive target for inhibiting HSC activation and the prometastatic liver microenvironment. These data have been published by a high-impact journal *Hepatology* (Wang Y. et al. *Hepatology*, 2019).

In addition, we tested the hypothesis that focal adhesion kinase (FAK) may regulate intracellular trafficking of TGFβ receptor II (TβRII) thereby promoting HSC activation. FAKY397F mutant and FAK inhibitor PF-573,228 were used to test this hypothesis. TβRII protein level was quantitated by immunoblotting and HSC differentiation into myofibroblasts was assessed by expression of HSC activation markers, alpha-smooth muscle actin, fibronectin, or CTGF. We found that targeting the kinase activity of FAK indeed suppressed TβRII protein level, TGFβ1-induced SMAD phosphorylation, and myofibroblastic activation of HSCs. At the molecular and cellular level, active FAK (phosphorylated FAK at Y397) bound to TβRII and kept TβRII at the peripheral plasma membrane of HSCs, and it induced TβRII phosphorylation at tyrosine 336. In contrast, targeting FAK or mutating Y336 to F on TβRII led to lysosomal sorting and degradation of TβRII. Additionally, we identified by RNA sequencing that the transcripts of 764 TGFβ targets were influenced by FAK inhibition and that through FAK, TGFβ1 stimulated HSC to produce a panel of tumor-promoting factors, including extracellular matrix remodeling proteins, growth factors and cytokines. Functionally, targeting FAK inhibited the tumor-promoting effects of HSCs *in vitro* and in a tumor implantation *in vivo* model. Thus, FAK regulates HSC activation by promoting TβRII to the plasma membrane and protecting TβRII from lysosome-mediated degradation. A manuscript about this project is currently under peer-review.

"Cancer invasion of the liver induces activation of hepatic stellate cells (HSCs), which are liver resident cells, into myofibroblasts and the activated-HSC/myofibroblasts in turn promote cancer implantation and proliferation in the liver. The bidirectional interactions between cancer and HSCs thus represent a therapeutic target for liver metastasis. My research program, funded by NIH R01 grant, focuses on identification of the molecular and cellular mechanisms governing HSC activation, which may lead to novel targets to inhibit HSC/cancer interactions and liver metastasis."

Ningling Kang



FROM LEFT TO RIGHT:
Front row: Qing Li, Ningling Kang, Donglian Liu
Back row: Xianghu Wang, Yuanguo Wang

Other professional activities:

Grant Review:
NCI Program Project (P01) Review, ZCA1 RPRB-F (O1), May 16-17, 2019

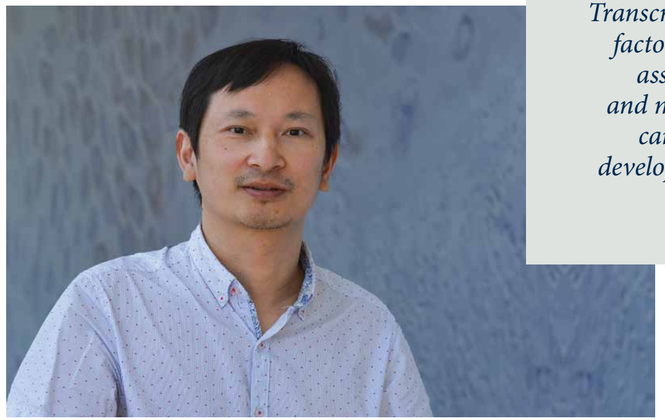
Presentations:
Oral Presentation at the Liver Meeting, AASLD, Nov. 2018: *Focal adhesion kinase promotes myofibroblastic activation of hepatic stellate cells by preventing lysosomal targeting and degradation of TGFβ receptor II*

Poster Presentation at the Liver Meeting, AASLD, Nov. 2018: *P300 acetyltransferase scaffolds with SMAD2/3 and TAZ to mediate their nuclear transport in TGFβ activation of hepatic stellate cells*

Transcription and Gene Regulation

Bin Liu, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR



"Transcription is the central step in the expression of genes, and a major mechanism for controlling the expression of a gene is to regulate its transcription. Transcription is regulated by various transcription factors. Many human diseases and disorders are associated with misregulation of transcription and mutations of transcription factors, including cancer, autoimmunity, neurological disorders, developmental disorders, diabetes, cardiovascular disease, and obesity."

Bin Liu

Summary

Our overall goal is to provide a structural basis of the initiation, elongation and termination of transcription and their regulations in bacteria. We shall study the formation of the transcription initiation complexes with various sigma factors, the transition from initiation to elongation and termination phases, the translocation of the RNA polymerase (RNAP) on template DNA, and the regulation of the RNAP by transcription factors at various stages of transcription. This objective will be achieved by determining the structures of the RNAP complexed with functionally associated proteins and appropriate DNA/RNA substrates captured at various steps in the processes, using cryo-electron microscopy and X-ray crystallography, as well as by appropriate biochemical experiments.

1. To elucidate the mechanisms of transcription initiation with alternative sigma factors and its regulation by activators

1.1 Transcription initiation with alternative sigma factors. In order to understand the underlying differences in promoter recognition and open complex formation by alternative sigma factors, we shall try to obtain structures of transcription initiation complexes with alternative sigma factors, including σ32, σ28, σ24 and σ19.

1.2 Regulation of initiation by activators. To enrich and advance our understanding of transcription regulation at initiation, we aim to obtain the structures of initiation complexes that include a bound general transcription factor, FNR protein, as well as other transcription factors (MerR-family proteins). The obtained information from this study would help us to understand how MerR-family members respond to the effector of xenobiotics and activate the transcription of multidrug resistance genes whose products are multidrug efflux pumps, which are one of three evolved antibiotic resistance mechanisms in bacteria.

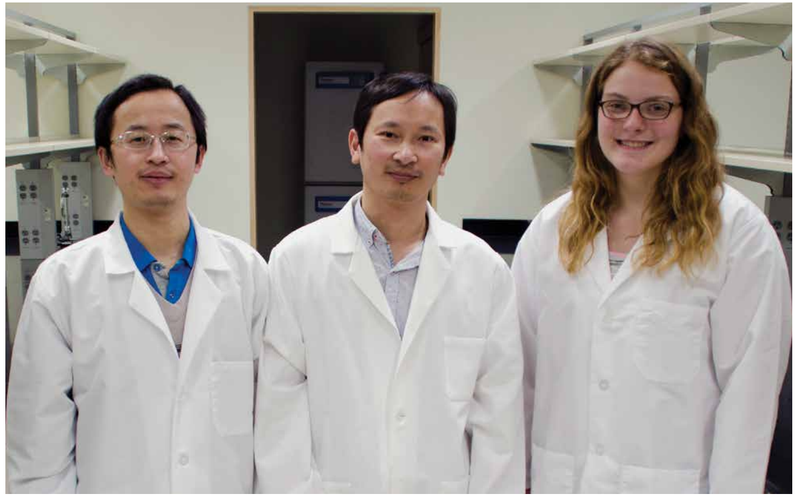
2. To understand the molecular basis for the regulation of transcription elongation by ATPases

In order to understand how ATPases regulate transcription reactivation, termination and transcription-associated DNA repair, we shall attempt to obtain structures of the RNAP elongation complexes in association with RapA and other ATPases, such as mfd and UvrD. We shall try to assemble and determine the structures of those complexes.

3. To characterize the basis of transcription termination and its regulation by the general transcription factors: NusA and NusG

3.1 In order to understand how intrinsic hairpin terminator induces transcription termination by a hyper-translocation mechanism, we shall try to obtain structures of the RNA hairpin-associated termination complexes. In addition, to understand how the transcription factor NusA enhances intrinsic termination, we shall also try to assemble and determine the structure of the intrinsic termination complex with NusA.

3.2 In order to understand how the hexameric helicase Rho binds to the rut site in the RNA transcript to terminate transcription, we shall try to obtain structures of the different complexes with or without the transcription factor NusG, which enhances this type of termination.

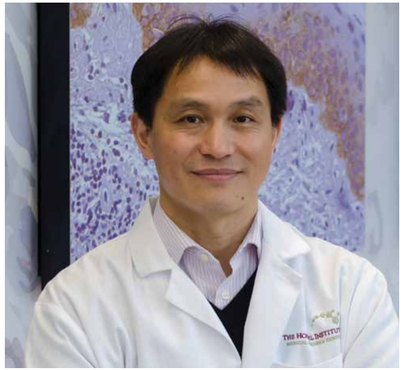


FROM LEFT TO RIGHT:
Wei Shi, Bin Liu, Abigail Schammel

Chromatin and Epigenetic Gene Regulation

Liang Liu, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR



Summary

Research in my lab focuses on the crosstalk between genetic and epigenetic factors that regulates skin homeostasis and carcinogenesis. We integrate epigenomics and functional genomics studies to better understand skin cancer development, and to identify novel actionable molecular targets for developing effective approaches to prevent and treat skin cancer.

Project 1. Molecular basis of UV-induced skin carcinogenesis, UV target gene identification and biomarker development for cancer prevention and treatment.

Despite decades of extensive research on UV and skin cancer, there remains a significant gap in our knowledge of the critical genes and pathways through which UV promotes skin carcinogenesis. In the past several years, my lab has conducted multi-omics studies to elucidate UV-induced genetic and epigenetic abnormalities in skin cells. Bioinformatics analysis have identified master regulators of skin UV response as actionable targets for developing mechanism-driven targeted approaches for skin cancer prevention and treatment.

Additionally, we have defined a novel UV gene expression signature (UVGES) panel consisting of conserved UV target genes. This UVGES panel has significant clinical potential because currently there is np sensitive molecular tests to quantify skin UV damage for cancer risk assessment. To validate the clinical utility of the UVGES panel, I have collaborated with former dermatologist colleagues at Columbia University Medical Center to collect skin biopsy and tumor tissues for gene expression analysis using the Nanostring nCounter platform. We have obtained interesting preliminary results showing that a subset of the UVGES genes are specifically associated with high-risk skin subtypes. The long term goal of this project is to develop a cost- effective biomarker-based test that can be easily adopted in clinical practice to risk stratify patients for targeted cancer prevention and treatment.

Research Interests:

- Epigenetic mechanisms in development and cancer pathogenesis.
- Role of hairless in epidermal homeostasis, immunity and carcinogenesis
- Novel molecular targets for cancer prevention and treatment

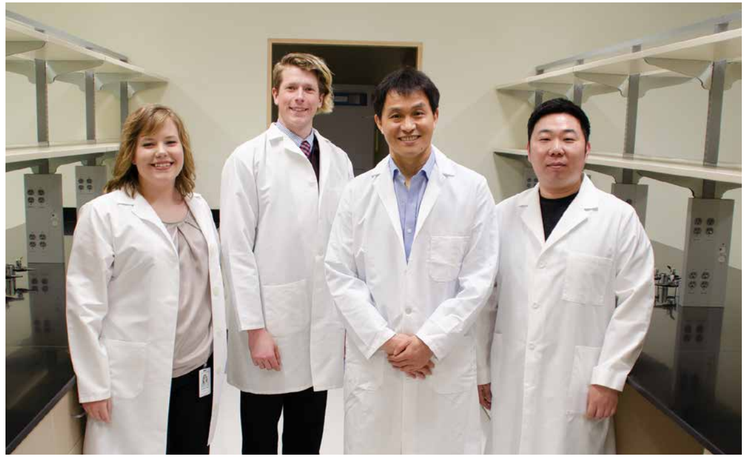
Project 2. Hairless transgenic *in vivo* models for studying skin homeostasis and immunity, epidermal stem cells and wound healing, and UV-induced tumorigenesis.

The hairless gene encodes a transcriptional co-regulator that is essential for skin homeostasis and hair follicle cycling. Several lines of evidence support the hypothesis that hairless is a master regulator of skin homeostasis via controlling the expression of target genes involved in cell proliferation, apoptosis, stem cell function and immune response. We recently reported that hairless is an epigenetic regulator with histone demethylase activity. Mutational inactivation of hairless alone can dramatically increase tumor incidence and burden in response to chemical induction or UV radiation. In human skin squamous cell carcinomas (SCCs) and other caner types, we found frequent deletions of the hairless gene locus. Moreover, hairless expression is frequently down-regulated in human SCCs but not actinic keratosis lesions. Through ChIP-seq studies, we have identified several hairless target genes that play important roles in cancer development (12). These findings strongly suggest that hairless is a pivotal tumor suppressor gene in skin carcinogenesis. We are following up these important findings to define the demethylase activity of hairless, its target genes, and the role of histone methylation in regulating epidermal homeostasis, stem cell activity and skin wound healing.

"Understanding the environmental origin of cancer and its underlying mechanism will facilitate the development of better cancer prevention strategies and targeted therapies."
Liang Liu

Patents:

- International Patent Application *"Next-generation biomarkers to detect sun damage and predict skin cancer risk"*
- International Patent Application *"UV biomarker gene subsets for developing novel diagnostic tools for risk stratification of cutaneous actinic keratosis and squamous cell carcinoma subtypes"*



FROM LEFT TO RIGHT:
Amanda Hinde, Michael Petersen, Liang Liu, Wootae Ha

To further define the role of hairless in skin homeostasis, we recently generated a K14-Cre driven epidermal-specific hairless knockout (HrΔ/Δ) *in vivo* model. Loss of hairless in the HrΔ/Δ skin led to a profound accumulation of dendritic epidermal γδ T cells (DETCs) coupled with upregulation of the IL-1/IL-36 family genes, suggesting that hairless may also regulate skin immunity. We are performing studies to elucidate the mechanism by which hairless regulates immune gene activity and to probe the role of IL-36 signaling in DETC activation and skin immunity. Ultimately, we aim to define the genetic and epigenetic pathways by which hairless modulates skin homeostasis and function and to identify new target genes that are critically involved in skin cancer development and immune regulation.

Memberships:

2010 - Society of Investigative Dermatology
2012 - New York Academy of Sciences
2019 - American Association for Cancer Research

Publications:

1. Shen et al., 2019. Identification of Master Regulator Genes of UV Response and Their Implications for Skin Carcinogenesis. *Carcinogenesis* 40:687-694
2. Queen et al., 2019. Function and regulation of IL-36 signaling in inflammatory diseases and cancer development. *Frontiers in Cell & Developmental Biology*, in press

Cancer Epigenetics and Experimental Therapeutics

Shujun Liu, Ph.D.

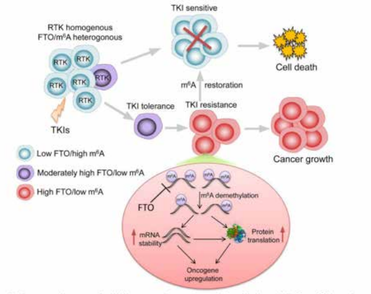
SECTION LEADER / ASSOCIATE PROFESSOR

Summary

Primary interests of our research section are to understand the molecular mechanisms and the roles of aberrant epigenetics (DNA methylation, histone protein modification, mRNA N^6-Methyladenosine) and protein kinase activity in cancer pathogenesis and drug resistance, and to translate the discoveries from bench to bedside by means of characterizing novel therapeutic reagents and developing innovative vehicles to efficiently and specifically deliver the drugs to the disease sites. In our laboratory, studies have included the causes of DNA hypermethylation and abnormal protein kinase activity, the mechanistic links between obesity and cancer, the dissection of molecular basis underlying resistance to molecular-targeted therapies and the development of innovative nanoparticles for drug delivery.

The role of mRNA N^6-Methyladenosine in leukemia resistance to kinase inhibitor therapy

Leukemia is an aggressive malignancy frequently associated with hyperactive tyrosine kinases (RTKs), such as BCR/ABL, c-KIT or FLT3 etc. Multiple RTK inhibitors (TKIs) have entered the clinic, but the rapid appearance of TKI resistance represent a major hurdle for successful leukemia treatment. While the acquired genetic mutations are the most extensively studied mechanisms of leukemia resistance to TKIs (i.e., imatinib, nilotinib), these genetic changes are insufficient to explain why the appearance of TKI resistance is relatively prompt upon drug exposure and the resistant phenotypes are partially reversible after a "drug holiday". In fact, recent studies have linked acquired TKI resistance to the dynamic variations in epigenome configurations in cancer cells. Yet the delineation of the key epigenetic events in TKI resistance is far from complete.

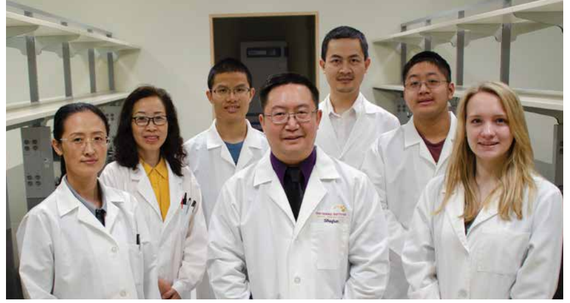


Schematic model illustrating the role of the FTO-m^{6}A axis in mediating the development of TKI resistant phenotypes

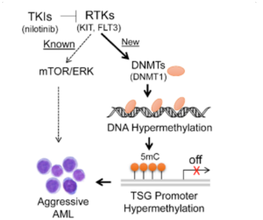
The N^6-methyladenosine (m^{6}A) is the most common epitranscriptomic modification on mRNA, which critically regulates gene expression at the post-transcriptional levels and displays dynamic and reversible features. We hypothesize that upon exposure to TKIs, the prompt and reversible nature of m^{6}A modification allows a set of proliferation/anti-apoptotic factors to be rapidly turned on, thus helping a subpopulation of cells escape TKI-mediated killing. To test this, we modeled and characterized TKI resistance in different leukemia models, including chronic myelogenous leukemia (CML) and acute myeloid leukemia (AML), and directly mapped m^{6}A in the transcriptomes of leukemia cells. We showed that the heterogeneous FTO-m^{6}A axis with the homogeneous RTK cells facilitates a subpopulation of cells to rapidly reprogram their m^{6}A methylome in order to upregulate survival and proliferation genes, so that they can withstand an initial onslaught of TKIs and continuously propagate in the absence of targeted kinase activities. On the other hand, the increased FTO-m^{6}A functions by TKIs further enhance expression of anti-apoptotic/survival genes, ultimately leading to the establishment of resistant phenotypes. When the FTO-mediated m^{6}A demethylation is either genetically or pharmacologically inhibited, the resistant cells regain partial sensitivity to TKIs. These findings have been published in a top journal, *Cell Research*.

Receptor tyrosine kinases are epigenetic activators in leukemia

Receptor tyrosine kinases (RTKs) are membrane-spanning proteins that exhibit intrinsic phosphotyrosine kinase activity. RTKs are frequently dysregulated in leukemia, yet the biological consequences of this dysregulation are largely unclear. The mechanisms underlying aberrant methylation, a hallmark of leukemia, are not fully understood. Further, because hyperactive RTKs crucially contribute to leukemia pathogenesis, their inhibitors (TKIs) have been broadly tested against leukemia. However, the molecular mechanisms by which TKIs suppress leukemia growth remain elusive. We found that upregulation of RTKs paralleled DNMT overexpression in leukemia cell lines and patient blasts. Knockdown of RTKs disrupted, whereas enforced expression increased, DNMT expression and DNA methylation. Treatment with the RTK inhibitor, nilotinib, resulted in a reduction of Sp1-dependent DNMT1 expression, the diminution of global DNA methylation and the upregulation of the p15INK4B gene through promoter hypomethylation in AML cell lines and patient blasts. This led to disruption of AML cell clonogenicity and promotion of cellular apoptosis without obvious changes in cell cycle. Importantly, nilotinib administration in *in vivo* model and human patients with AML impaired expression of DNMTs followed by DNA hypomethylation, TSG re-expression, and leukemia regression. Our study provides the first evidence that RTKs are modulators of DNMT1-dependent DNA methylation in leukemia cells. Our study has for the first time documented that TKIs impair DNMT1 expression resulting in global and gene specific DNA hypomethylation. These findings demonstrate RTKs as new types of epigenetic regulators and unravel a signaling interaction between RTKs and DNMTs in leukemia pathogenesis, shedding light on leukemia molecular biology. Our data identify the DNA hypomethylating activities of TKIs, thus significantly expanding the pool of DNA methylation inhibitors. Our discoveries provide a mechanistic explanation why TKIs show therapeutic efficacy in patients without target mutations, and suggest that altered DNA methylation profile might be alternative predictors of responses in patients without RTK mutations. Altogether, our work provides the preclinical rationale for using TKIs to benefit patient subpopulations characterized by aberrant DNA methylation including those who relapse from current epigenetic therapy.



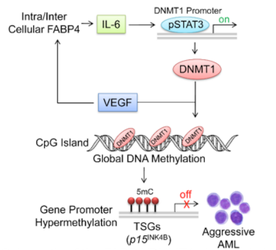
FROM LEFT TO RIGHT:
Yanhong Tan, Jiuxia Pang, Tao Cheng, Shujun Liu, Ning Xiang, Hao Liu, Katie Waller



Schematic model illustrating the DNA hypomethylating potential of nilotinib

The FABP4-DNMT1 loop is a new epigenetic target for cancer therapy

Acute myeloid leukemia (AML) is a highly aggressive hematologic malignancy characterized by the swift uncontrolled growth of immature myeloblasts. It is a lethal disease that lacks effective treatment. Although the precise molecular causes that are responsible for AML development and disease progression are unclear, it seems to result from an interplay of genetic and environmental factors that are largely unidentified. Aberrant DNA methylation mediated by dysregulation of DNA methyltransferases (DNMT) is a key hallmark of AML, yet efforts to target DNMT dysregulation for drug development have lagged. We previously demonstrated that upregulation of fatty acid-binding protein 4 (FABP4) promotes AML aggressiveness through enhanced DNMT1-dependent DNA methylation. Here we demonstrate that FABP4 upregulation in AML cells occurs through vascular endothelial growth factor (VEGF) signaling, thus elucidating a crucial FABP4-DNMT1 regulatory feedback loop in AML biology. We show that FABP4 dysfunction by a selective inhibitor BMS309403 leads to down-regulation of DNMT1, decrease of global DNA methylation and re-expression of p15INK4B gene by promoter DNA hypomethylation in vitro, ex vivo and in vivo.



Schematic of the FABP4/DNMT1 vicious circle in aggressive AML leukemia

Overall, our discoveries offer new insights into the molecular biology of cancer pathogenesis and drug resistance, advance our understanding of nanoscience with efficient delivery vehicle for miRs and small molecule compounds, and foster the translation of nanotechnology solutions to biomedical applications thereby improving the management of cancerous lesions.

Cryo-EM and Molecular Cell Biology

Anna Sundborger-Lunna, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

In the Cryo-EM and Molecular Cell Biology Section we are studying how membrane-remodeling proteins assemble into large complexes and regulate fundamental cell events, such as apoptosis, autophagy and mitochondrial dynamics. Our current focus is to determine how dysregulation of mitochondrial dynamics contributes to infectious diseases and cancer (**Fig. 1**). Several molecular players with membrane-remodeling activity have been implicated in mitochondrial dynamics: (i) a potential tumor suppressor involved in a multitude of intracellular membrane trafficking events; (ii) dynamin family member Drp1, which is required for fission of the mitochondrial outer membrane (MOM); (ii) Immunity-Related GTPase M (IRGM), an GTPase related to the dynamin super family critical for autophagy during the innate response to intracellular pathogens; and (iii) Bax, a pro-apoptotic protein that trans-locates to the mitochondria where it is activated to form pores in the MOM, leading to the release of cytochrome c and subsequently, apoptosis.

Our previous data show that neuronal-specific endophilin isoform, endophilin A1, is critical for recruitment and assembly of dynamin 1 at sites of plasma membrane fission (1).

"Cryo-EM allows us to visualize exactly how critical cellular events, such as membrane remodeling and tubulation are initiated and regulated by BAR proteins."

Anna Sundborger-Lunna

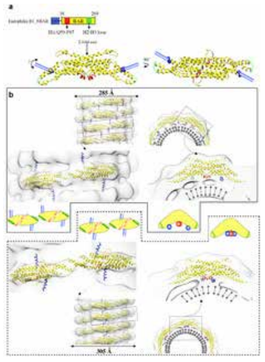


Figure 1. ***Mitochondrial dynamics***. Mitochondrial membrane-remodeling events, including fission fusion, mitophagy and apoptosis are controlled by members of the dynamin super family of large atypical GTPases, Drp1, Mitofusin, OPA1, and IRGM. Image modified from original (1)

Bob Ashley is the Electron Microscopy Core Facility manager. He is a graduate of Madison College in Madison Wisconsin, which at the time, was one of two professional schools in the nation with a program focused solely on electron microscopy and electron optics. His first position was at the Penn State College of Medicine in Hershey Pennsylvania and received further training at The University of Pittsburgh as an apprentice in the craft of cryo-EM. After 6 years developing a biological cryo-EM core at Penn State, he came back to the Midwest when the Hormel Institute recruited him to run their premier Electron Microscopy facility and has been here since August of 2016.

Bob Ashley, Cryo-EM Manager

FROM LEFT TO RIGHT:
Veer Bhatt, Anna Sundborger-Lunna

Current research projects:

1) PROJECT 1: Membrane shape controls neuronal cell death
2) PROJECT 2: Coordinated regulation of Golgi membranes remodeling controls autophagosome formation
3) PROJECT 3: Endophilin B1-mediated membrane remodeling controls Bax-dependent membrane permeabilization and cell death
4) PROJECT 4: ABHD5-mediated membrane remodeling controls PNPLA2-controled lipolysis
5) PROJECT 5: Innate immunity protein IRGM controls mitochondrial membrane fragmentation and xenophagy

Other professional activities:

Invited Speakers:

- Department of Pediatrics
  SANFORD Health, Sioux Falls, SD
  2019
- Department of Biological Sciences
  Minnesota State University, Mankato, MN
  2019
- Center for Molecular Medicine and Genetics
  Wayne State University, Detroit, MI
  2018

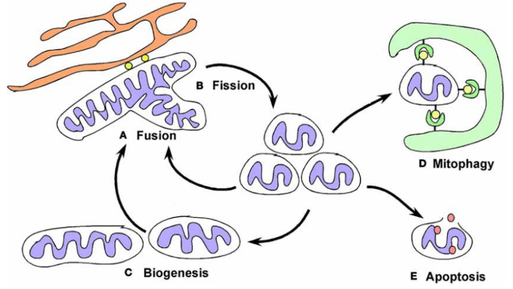
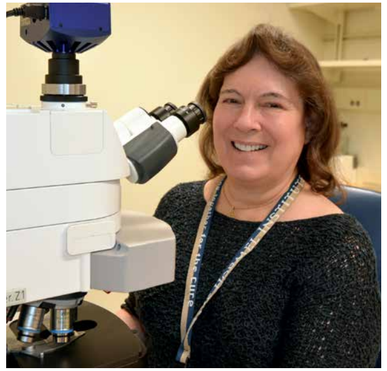


Figure 2. ***Cryo-EM three-dimensional (3D) reconstructions of endophilin B1 helical scaffolds assembled on lipid tubes***. (a) Endophilin B1 protein domain organization (top panel), and atomic model based on cryo-EM map and homology modeling (I-TASSER) (bottom panel). Arrows denote putative orientations of N-terminal amphipathic helix, H0. (b) Cryo-EM maps of endophilin B1-decorated lipid tubes with outer diameter of 28.5 nm (top panel) and 30.5 nm (bottom panel) with atomic model positioned into protein density by docking. Cartoons illustrate the alternate positions of H0 (blue) and H1i (red). Note the orientation of H0 and H1i proximal to the lipid bilayer (grey).

Stem Cells and Cancer

Rebecca J. Morris, Ph.D.

SECTION LEADER / PROFESSOR



Summary

The principal techniques we use are 1) advanced light microscopy of cells and tissues, and 2) fluorescence activated cell sorting of epidermal cells, cells from skin tumors, and blood and bone marrow cells. These methods enable us to determine the roles of bone marrow cells in epithelial cancers. We perform quantitative histopathology on sections of skin tumors to determine the interaction of cells from the bone marrow with the epidermis. We also use next generation RNA sequencing to determine the molecular signatures of epidermis and bone marrow derived cells.

"We visualize how cancer happens by determining how stem cells behave, how they move around the body, and how they develop into tumors."

Rebecca J. Morris

Non-melanoma skin cancers such as basal cell and squamous cell carcinomas occur more frequently in the human population than any other type of malignancy, and more than one million new cases are diagnosed in the United States annually. It is estimated that one-third to one-half of all human cancers originate in the skin; that skin cancers exceed all others combined; and that the lifetime risk for development of skin cancer in the US population is one in five. Solar ultra-violet radiation is the major known cause of non-melanoma skin cancers and is directly relevant to the etiology of skin cancer, as demonstrated by both epidemiological evidence and the tight correlation between non-melanoma skin cancer development in humans and ultra-violet radiation-induced skin carcinogenesis in *in vivo* experimental models.

Skin cancers as seen in the clinic are actually the results of a long history of which only the later stages are easily observed. The progression from normal maintenance of the skin to neoplastic growth involves multiple changes in cellular phenotypes and patterns of gene expression.

Cells covering surfaces such as the epidermis or lining spaces like the gastrointestinal and respiratory tracts are called epithelial cells, and can be identified due to their genetic expression of certain cytoplasmic filaments called keratins or to a cell surface marker such as epithelial cell adhesion molecule (EpCAM). Additionally, these cells can be tracked as they move around the body since a green fluorescent protein (GFP) under the control of cytokeratin promoters is available. This enables functional studies because we can use the GFP to visualize, isolate, and culture these cells to investigate their stem cell epithelial origin.

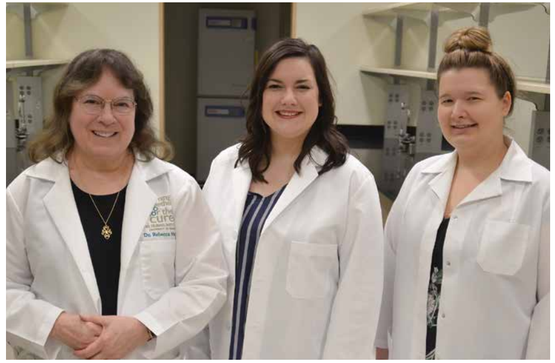
There are several reports of cells with epithelial markers in blood and bone marrow of "normal, healthy human subjects"; however, the proliferative and differentiative capacities of these cells are to this date unknown. These bone marrow derived epithelial cells are heterogeneous with regard to their repertoire of surface markers and cytoplasmic cytokeratin expression. For the most part, they are considered "undesirable background" in the "liquid biopsy" that seeks to measure circulating metastatic cells in blood and bone marrow of cancer patients. Nevertheless, although their presence cannot be denied, their functional and phenotypic characteristics and other attributes are unknown.

Several attributes of blood borne epithelial cells have been associated with epithelial cancers. First, we know that the incidence of circulating epithelial cells with characteristics of cancer cells increases with metastatic disease. Second, Houghton and colleagues demonstrated that some bone marrow derived cells are recruited to and contribute to tumors in the Helicobacter model of *in vivo* gastric cancer. Third, Pantel and associates showed that circulating epithelial cells increase in some benign proliferative diseases of the colon. Moreover, Tamai and colleagues have demonstrated that some bone marrow derived cells are recruited to the epidermis in an *in vivo* model of epidermolysis bullosa. Further, we have recently demonstrated that bone marrow derived cells contribute to both initiation and promotion of cutaneous neoplasms (Park, Morris, and associates). These observations taken together lead to the conclusion that is essential to determine the proliferative and differentiative properties of the circulating epithelial cells.

Thus, bone marrow derived epithelial cells might be a reserve population of progenitors. For sure, they are recruited to cutaneous papillomas and non-healing ulcers. For sure, carcinogen-exposed bone marrow can initiate benign and malignant cutaneous neoplasms upon skin tumor promotion. But, beyond carcinogenesis, do they play a role in chronic wound healing? Do they actually have properties of stem cells?

We began to lay the groundwork for this proposed research upon reading the paper by Houghton et al., from the laboratory of T.C. Wang who reported that bone marrow derived cells contributed to gastric cancer in the *in vivo* Helicobacter model. We then demonstrated that bone marrow cells also contributed significantly to non-melanoma skin cancer, and in addition, proliferated, produced epidermal keratins, and stratified as in epidermal terminal differentiation. We also demonstrated that carcinogen-exposed bone marrow contains cells capable of becoming tumor-initiating cells. A critical unanswered question from this work is: what is the nature of the recruited cells? No defining functional studies addressing this hypothesis have been forthcoming. Such information would be exciting.

We used gender-mismatched bone marrow transplantation in the context of a skin carcinogenesis to determine the recruitment of bone marrow cells in skin tumors. We detected clusters of five or more bone marrow cells in over 40% of benign papillomas where they occupied 25% or more of the lesional area. The bone marrow cells clustered in the cutaneous epithelium where they expressed skin keratins, and proliferated and stratified, and contributed to the lesions along with the progeny of the hair follicle stem cells, but as separate populations. We also performed cell culture experiments where we cultured bone marrow adherent cells in the presence of filter-separated epidermal keratinocytes and found that the presence of keratinocytes induced some of the bone marrow cells to make epidermal keratins. Further, after bone marrow transplant from donors previously exposed to a carcinogen, several benign papillomas and a squamous cell carcinoma were observed after exposure to a skin tumor promoter alone.



FROM LEFT TO RIGHT:
Rebecca Morris, Stephanie Holtorf, Caitlin Lura

Other professional activities:

Park H., Lad, S., Boland, K, Johnson, K., Readio, N., Jin, Guangchun , Asfaha, S., Patterson, K.S., Singh, A., Yang, X., Londono, D., Singh, A., Trempus, C., Gordon, D., Wang, T.C., and **Morris, R.J.** Bone marrow-derived epithelial cells and hair follicle stem cells contribute to development of chronic cutaneous neoplasms. Nature Communications (2018). https://doi.org/10.1038/s41467-018-07688-8.

We conclude that surprisingly large numbers of bone marrow cells are recruited to a subset of cutaneous papillomas and non-healing ulcers and reflect a previously unrecognized systemic contribution to these lesions. We also conclude that carcinogen-exposed cells originating in the bone marrow can initiate skin tumors in the presence of a skin tumor promoter that by itself does not cause cancer. Ultimately, these findings may aid discovery of new methods of preventing, diagnosing, or treating non-melanoma skin cancer, and may provide a new source of progenitor cells for use in regenerative medicine.

This research is significant because it will have far-reaching consequences for fields of hematology and epithelial biology. First, we will document and quantify bone marrow derived epithelial cells in healthy subjects to determine the phenotypes in humans the phenotypes expressed. Second, we will determine whether the bone marrow derived epithelial cells have functional characteristics of epithelial stem/progenitors in culture and *in vivo* models. The experiments outlined here have the potential to open up a new field of research in epithelial biology and hematology and should provide motivation for development of new disease models. The implications of our findings toward understanding the etiology of epithelial cancers in general as well as their diagnosis and treatment have not escaped our notice.

Cell Signaling and Tumorigenesis

James Robinson, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

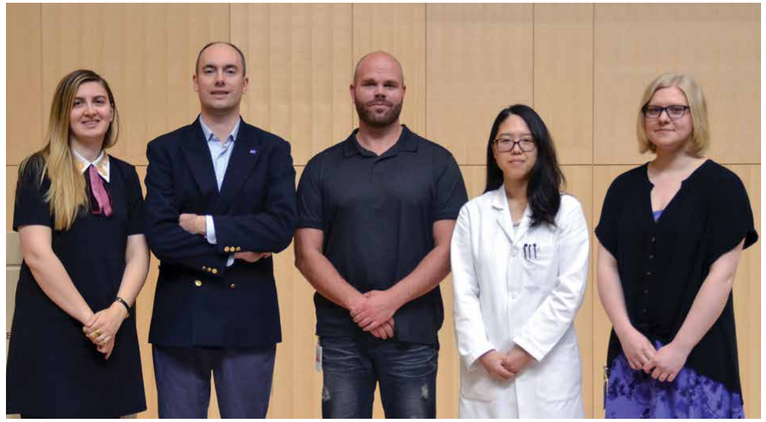
Summary

Our section is concerned with the molecular mechanisms by which oncogenic signaling regulates Tumorigenesis, with the ultimate goal of developing and improving existing therapeutic approaches to eliminate cancer. As part of the University of Minnesota and a member of the Masonic Cancer Center (MCC), have and will continue to collaborate with worldwide experts in the fields of cell signaling, cancer research comparative pathology and genetics. We employ two experienced postdoctoral fellows, Florina Grigore M.D., and Hana Yang, Ph.D, and Nick Hanson a technician and Caoimhe Farrell an Undergraduate Research Experience (SURE) intern. Hana has successfully completed her postdoctoral training at the Hormel Institute and starts a new phase of her training at the Mayo Clinic in Rochester Minnesota this fall.

Melanoma: Melanoma is the most dangerous form of skin cancer. According to the CDC, Minnesota has the highest incidence of melanoma in Midwest states. Despite advances in melanoma research, the five-year survival rate for patients with advanced Melanoma remains around 16%. Two therapeutic agents, vemurafenib, and dabrafenib, are FDA-approved for the treatment of advanced melanomas that carry BRAFV600E mutations. Although the initial response to these inhibitors can be dramatic, the melanomas nearly always become resistant. Using a novel *in vivo* model of melanoma we have developed that provides an exceptional experimental system to study this issue. We are determining and validating the mechanisms of melanoma's resistance to these inhibitors. This work is generously funded through an American Cancer Society Research Scholar Grant worth nearly $800,000. The American Cancer Society is the largest nonprofit source of grant funding for cancer researchers in the United States, other than the government.

Glioma: Gliomas are the most common primary brain tumor. Glioblastoma (GBM), the highest grade of glioma (most lethal), is highly infiltrative, and is resistant to all conventional therapies. Patients with this cancer rarely survive longer than 12-14 months from the time the tumor is diagnosed. Pediatric GBM is clinically and biologically distinct from the adult disease. It typically develops in the midline or pons. While even the lowest grade of glioma in children Pilocytic astrocytoma is associated with significant morbidity diffuse intrinsic pontine glioma (DIPG), a GBM of the brain stem confers the worst prognosis of any pediatric cancer. It has a 5-year survival rate of <1%, a 1-year survival of <30% and 2 -year survival of <10 %; median survival is < 9 months. Pediatric GBM is defined by mutations in the gene encoding Histone H3.3. We are developing an animal model (Figure 1) to study this disease. In collaboration with the Hinchcliffe lab at the Hormel Institute, we seek to bring about a better understanding of the role of this mutation in these tumors in order to develop new therapies to improve survival for children with this devastating disease.

Colon Cancer: After lung and prostate cancer, colon cancer the leading cause of cancer deaths in the United States with 132,770 new cases and 49,700 deaths anticipated in 2015. After lung and prostate cancer, colon cancer the leading cause of cancer deaths in the United States with 132,770 new cases and 49,700 deaths anticipated in 2015. About 75% of cases are sporadic with no obvious evidence of an inherited disorder. The remaining 25% of patients have a family history of CRC that suggests a hereditary contribution, common exposures among family members, or a combination of both. Familial adenomatous polyposis (FAP), is one of the most clearly defined and well understood of the inherited colon cancer syndromes. Our preliminary data has demonstrated that loss of APC is insufficient for tumorigenesis and additional growth signals or mutations are also required for nuclear accumulation of β-Catenin and intestinal polyposis. Since *in vivo* models of FAP develop a multitude of intestinal polyps without additional genetic alterations, these additional signals are likely to arise from adjacent stromal cells. If we can show that stromal signaling plays a driving role in tumorigenesis, following or pre-empting epithelial LOH of APC, it should be possible to develop targeted therapeutics to block this signaling. A major preliminary finding is that heterozygous mutation of APC in adult *in vivo* model is not sufficient cause tumor formation. Our sections work on Colon cancer has been funded by the National Institutes of Health (NIH) and our ongoing studies will contribute to the development of novel therapies and improve the outcome for patients with colon cancer.



FROM LEFT TO RIGHT:
Florina Grigore, James Robinson, Nick Hanson, Hana Yang, Caoimhe Farrell

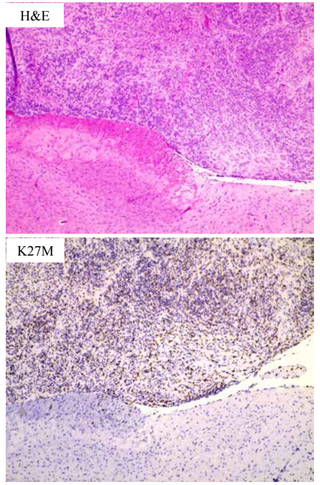


Figure 1. Histology using hemotoxylin and eosin (H&E) staining shows ventral brainstem location of tumor. High magnification (200×) H&E image reveals high-grade tumor characteristics (pseudopalisading necrosis, mitotic figures, neovascularization), and immunohistochemistry image shows the HA-tagged H3.3K27M (nuclear) expressing cells.

Computational Cancer Genomics

Rendong Yang, Ph.D.

SECTION LEADER / ASSISTANT PROFESSOR

Summary

Developing novel algorithms for indel detection. Our most recent work has been in the development of clinical genomic variant detection pipelines for our customized oncology gene panels in the University of Minnesota Molecular Diagnostic Lab. Briefly, we developed new algorithm named ScanIndel to accurately detect insertion and deletion (indel) mutations in human genome from next generation sequencing data. In particular, ScanIndel reliably detects medium-size and large indels. With this method, indels contribute to pathogenesis of constitutional and somatic diseases can be identified quickly and accurately which is important for targeted therapy or patient prognosis.

Detecting gene rearrangement, splicing and epigenetic regulator of AR in prostate cancer. We developed an integrated pipeline to detect structural rearrangements in the AR gene, which encodes the androgen receptor. Through this work, we identified diverse genomic-dependent and genomic-independent AR splicing variants expressed prostate cancer. Additionally, our early work focuses on prostate cancer eipgenomic studies by analyzing the ChIP-seq data of AR and BRD4 in prostate cancer VCaP cell line. Our study revealed the crosstalk between AR and BRD4 signaling in prostate cancer progression.

Developing algorithms for detecting cancer biomarkers. We have been developing EgoNet algorithm by integrating gene expression microarray data and protein interaction networks to identify network modules that can distinguishing different breast cancer subtypes.

1. Detecting novel indels from prostate cancer genome

We developed transIndel, a splice-aware algorithm that parses the chimeric alignments predicted by a short read aligner and reconstructs the mid-sized insertions and large deletions based on the linear alignments of split reads from DNA-seq or RNA-seq data. TransIndel exhibits competitive or superior performance over eight state-of-the-art indel detection tools on benchmarks using both synthetic and real DNA-seq data. We applied transIndel to DNA-seq and RNA-seq datasets from 333 primary prostate cancer patients from The Cancer Genome Atlas (TCGA) and 59 metastatic prostate cancer patients from AACR-PCF Stand-Up- To-Cancer (SU2C) studies. TransIndel enhanced the taxonomy of DNA- and RNA-level alterations in prostate cancer by identifying recurrent FOXA1 indels (**Figure 1**).

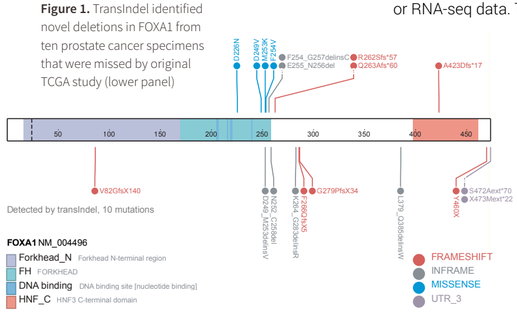


Figure 1. TransIndel identified novel deletions in FOXA1 from ten prostate cancer specimens that were missed by original TCGA study (lower panel)

"Identifying specific subtypes of prostate cancer and the distinct pattern of mutations associated with them will enhance development of precise diagnostic tools that detect specific genetic aberrations, allowing doctors to reliably predict a patient's outcome and prescribe personalized treatment."

Rendong Yang

2. Delineating lncRNA landscape in prostate cancer genome

Prostate cancer (PCa) is the most commonly diagnosed cancer in men in United States, with significant health impact. Clinically, it is complicated with the lack of biomarkers and effective treatments for aggressive disease, particularly castration-resistant prostate cancer (CRPC). We have gained much insight into the biology of PCa through studying protein-coding genes, but they represent only a small fraction of our genome. It is now well accepted that the vast majority of human genome (about 75%) is actively transcribed, but protein-coding genes only account for about 2% the genome. This means the majority of the human transcriptome is comprised of noncoding RNAs (ncRNAs). Among ncRNAs, long noncoding RNAs (lncRNAs), typically >200 bp, commonly characterized by polyadenylation, splicing of multiple exons, promoter trimethylation of histone H3 at lysine 4 (H3K4me3), and transcription by RNA polymerase II, have increasingly been recognized as playing essential roles in tumor biology, representing a new focus in cancer research.

Many lncRNAs have been shown to be either up- or download-regulated in various cancers, including PCa. Several PCa-specific or PCa-associated lncRNAs have been identified to date, but only a few have been validated in independent patient cohorts or approved for clinical practice. Our current research is developing novel computational methods to achieve the first complete compendia of CRPC-associated lncRNAs and reveal the dynamic interplay between lncRNAs and tumorigenesis, progression and metastasis, which will highlight the importance of lncRNAs in the etiology of PCa.

3. Detecting novel biomarkers for cancer immunotherapy

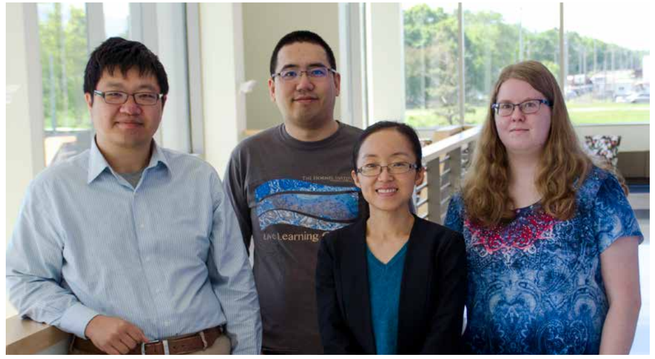
Immune checkpoint blockade therapy has proved to be effective on a number of cancer types such as skin, lung and kidney cancer. However, only part of the patients has response to immunotherapy drugs. We have developed a novel computational algorithm which can sensitively detect previously missed novel splicing events in human transcriptome from RNA-seq data. We utilize whole exome sequencing and RNA-seq from renal cell carcinoma, lung cancer and melanoma to correlation of the expression of our detected splicing event with immune checkpoint therapy response or resistance. This study aims to improve the computational methodology to detect and quantify novel alternative splicing events and to determine their involvement in immunotherapy-associated phenotypes. Integrative analysis of DNA mutations and RNA splicing events in the responders and non-responder patients is able to identify a list of candidate genomic independent alternative splicing events that play a role underlying the resistance of immunotherapy in the non-responders or the effects in the responders.

Other professional activities:

Award:
DoD Idea Development Award 2018

Publication:
Wang TY, Wang L, Alam SK, Hoeppner LH, **Yang R.** ScanNeo: identifying indel-derived neoantigens using RNA-Seq data. Bioinformatics. 2019 Mar 18.

FROM LEFT TO RIGHT:
Rendong Yang, Tingyou Wang, Yanan Ren, Emma Rector



SUPPORTING DEPARTMENTS

FROM LEFT TO RIGHT:
Nate Britt, Duane Graff, Mark Severtson, Randy Johnson
Not Pictured: Brandon Hoium, Kim Downey

Building Operations and Maintenance

Mark Severtson / Building Systems Manager

The Maintenance, Buildings and Grounds team to work with all the current departments and new staff coming in to keep the facilities and research running smoothly, keeping the building running as safe, efficient, and as beautiful as possible. We work with new researchers to set up labs as needed, along with any new equipment that arrives. This department continually makes improvements to keep the facility running as efficiently as possible. We are also part of the Energy Hero program with the Austin Utilities to help reduce power usage when there is a peak alert day.

Instrument Core Facility

Todd Schuster / Core Facility Manager

The Shared Instrument Core operates and maintains the shared instruments at the Hormel Institute. We also instruct scientists in the use of these instruments and ensure that they receive the proper maintenance. New instruments added to the Core this year include the BD Fortessa X-20 flow cytometer. With more excitation lasers and more detectors the Fortessa offers greater fluorophore flexibility and higher resolution than our previous analyzer. Other additions include a Leica TP 1020 Tissue processor and a Leica HistoCore Arcadia embedder. These devices allow users to prepare tissue samples for mounting on slides. Our Core also includes several microscopes, a Zeiss Apotome which allows for a wide variety of fluorophore options and the ability to eliminate out of focus light with internal grids. Other microscopes include a Zeiss Palm Microbeam laser dissection microscope which can be used to isolate high-purity tissue from cryosections and FFPE samples and a Nikon C1 confocal system. Recent additions include the Biacore T200 which uses surface plasmon resonance technology to measure interactions between proteins, lipids, and small molecules, and the IncuCyte S3 Imager which allows researchers to perform real time live cell analysis. Other shared instruments include a Becton Dickinson FACS ARIA II cell sorter, ABSCIEX 5600 Triple TOF mass spectrometer with an Eksigent Nano LC system, and a Rigaku X-Ray diffraction system for protein crystallography. A Bio-Tek Synergy plate reader, real time qPCR instruments, chemiluminescence imagers, spectrophotometers, and centrifuges are also available for use. Histology equipment consists of a microtome and cryostat in addition to the newly added equipment listed above. Ultimately, we look forward to employing these and other core facility equipment to assist research at The Hormel Institute.

FROM LEFT TO RIGHT:
Todd Schuster, Josh Monts

Library

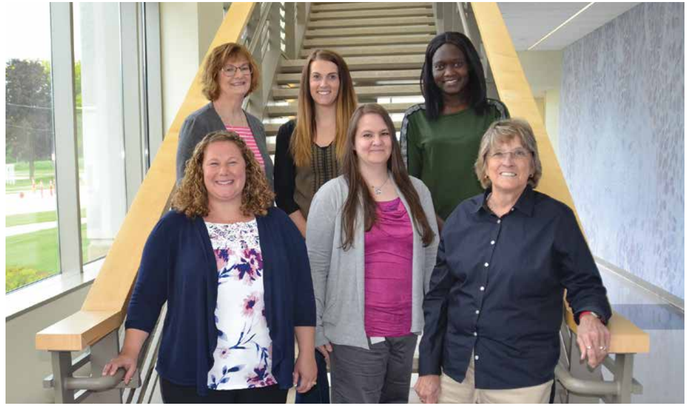
Andy Lucas / Librarian

The library serves as the information resource center for The Hormel Institute. It provides print and online materials to support faculty and staff research as well as special projects. The collection consists of approximately 10,250 volumes of bound journals and 2,900 volumes of books and serials. The subjects covered include chemistry, biology, biophysics, medicine, and electronics. Researchers also have access to the services and resources of the University of Minnesota Twin Cities Libraries, the 15th largest in North America by collection size. Books are delivered from the Twin Cities campus and are available for pickup in The Hormel Institute Library. Articles that are not available in electronic form are obtained through interlibrary loan.

Administration

Ann M. Bode, Ph.D. / Interim Executive Director

The administrative team continues to expand as we strive to provide the highest level of clerical, account management, acquisition, compliance and human resource assistance to our research and support sections. Additionally, Administration works closely with the University's central administration departments, serving as liaison between the two entities.



FROM LEFT TO RIGHT:
Front row: Minda Anderson, Stephanie Blaser, Dr. Ann M. Bode
Back row: Julie Gerstner, Becky Earl, Nyayow Dong

Research Support Group

Kim Klukas / Supervisor

The Hormel Institute's Research Support Group is continually striving to provide top notch services to the institute's investigative staff. This past year team members attended national meetings, statewide seminars, and online short courses to finely tune their skill sets. Two team members are currently working towards becoming certified within the industry which will help to increase their specific knowledge and abilities required for maintaining high level research support. The team works hard to provide leading edge training and assistance to ensure high quality research here at The Hormel Institute.

FROM LEFT TO RIGHT:
Front row: Chrissa Campbell, Kate Viker, Kim Klukas
Back row: Chelle Jacobson, An Le

FROM LEFT TO RIGHT:
Mike Conway, Jeffrey McDonald, Theresa Tucker, Tim Lastine

Research Support Services

Jeffrey McDonald / RSS Manager

Research Support Services continues to provide instrument maintenance along with computer, telecommunication, network, and internet support for The Hormel Institute. Maintenance includes a wide variety of scientific instruments, from complex to simple and large to small. Computers and network connectivity are an extremely important resource for researchers and a major portion of our work load. As always, the network security needs keep us busy. The Linux cluster CAL42 (Computational Analysis of Life-sciences 42) has been calculating away simulating protein molecules in our supercomputer room, part of The Hormel Institute's International Center of Research Technology.

DEVELOPMENT AND PUBLIC RELATIONS

Gail Dennison, M.A., CFRE

DIRECTOR OF DEVELOPMENT AND PUBLIC RELATIONS

FROM LEFT TO RIGHT: Brenna Gerhart, Kathi Finley, Gail Dennison, Gretchen Ramlo, Michelle Hjelmen

Our department's guiding mission is to gain support for the cancer research of The Hormel Institute, University of Minnesota. As the Institute's scientists seek meaningful discoveries in the quest to prevent and control cancer, our goal is shine the light on their research and find support so even more research can be achieved and more answers accelerated. We consider it a privilege to work with these expert researchers from around the world - they have dedicated their lives to research with the broad goal of furthering knowledge so people can live longer, healthier lives, uninterrupted by cancer. To all who contribute so generously and support the research of The Hormel Institute, thank you. Your faith and support indeed makes an important difference.

This year, over one thousand individuals and businesses gave to The Hormel Institute University of Minnesota through monetary donations. Thanks to The Hormel Foundation, every penny of each donation to The Hormel Institute directly funds research – none to administrative costs.

In the past year, donations have funded new technology, 17 seed grants, and projects in new cancer research areas. Donations allow our scientists to pursue research that wouldn't otherwise be possible and can lead to new discoveries and increased funding.

Thank you from one and all at The Hormel Institute, University of Minnesota.

The Hormel Foundation
University of Minnesota
Masonic Cancer Center, University of Minnesota
Hormel Foods
Mayo Clinic
Eagles 5th District Cancer Telethon
Lyle Area Cancer Auction
Paint the Town Pink
Plunging for Pink
Fishing for a Cure
Bowling for the Battle
Karl Potach Foundation
Lynch Livestock Inc.
Dutchtown Jumpers
Austin Bruins
Gary and Pat Ray
Dick and Diane Gerhardt
Renewable Fuels Foundation
Kansas Corn
Tom and Lori Day
Joel and Beth Johnson
Judi and Dick Bergen
Blooming Prairie Cancer Group
Pink Pumpkin Patch Foundation
Absolute Energy LLC
Compeer Financial
Iowa Renewable Fuels Association
Reichel Foods
Mower County Fair Board
Mower County Habitat and Pheasants Forever – Hunt for a Cure
Ollie and Shar Hagen
American Coalition for Ethanol
Iowa Renewable Fuels Association
Minnesota Corn Growers Association
Greater Mower County Paint the Town Pink
Governor Tim Walz
State Senator Dan Sparks
State Senator David Senjem
State Representative Jeanne Poppe
SKB Environmental
Waste Connections
Chad Thomas – Thrivent Financial
Martha Vrieze – Thrivent Financial
Hy-Vee Austin
KAAL – ABC 6 News
KAUS Radio
Discover Austin
Richard and Nancy Knowlton
City of Austin
GRAUC – Greater Rochester Advocates of Universities and Colleges
Speakers Assembly of Southwest Florida

THANK YOU

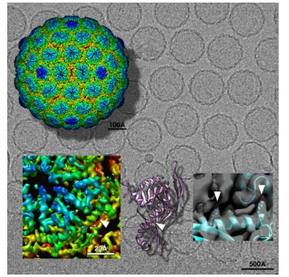
for moving research forward

PARTNERS IN GROWTH

Every Dollar

Our donors make a difference. Nearly 17 innovative research projects are underway that would not be possible without your donations. The studies may be targeted for specific cancers - such as breast, prostate or Wilms' Tumor cancers - but the knowledge gained about the initiation and progression of cancer can impact understanding of many cancers. Thank you for joining our mission with your donations - together we will accelerate answers to cancer so people live longer, healthier lives.

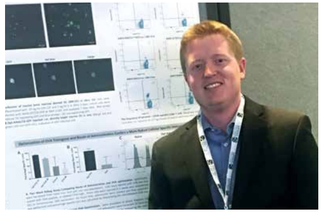
LYNCH Family FOUNDATION

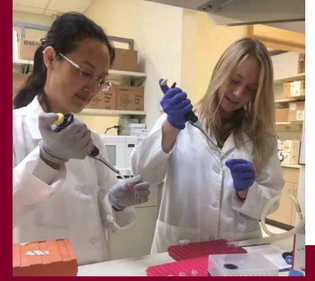


Our goal at The Hormel Institute is to intervene, prevent and control cancer at its earliest possible detection. You - our donors - provide the support that keeps our research moving forward toward achieving that goal. Annual fundraisers, individuals and families, community groups, sports teams, and corporate donors like Compeer Financial, Absolute Energy, and Hormel Foods are all part of how we work together. Thanks to The Hormel Foundation, 100% of every donation funds our innovative, world-recognized cancer research. Thank you for your support of The Hormel Institute - through your support, you are helping create a world where people live longer, healthier lives.

Furthers Answers

Today's EDUCATION, Tomorrow's DISCOVERIES

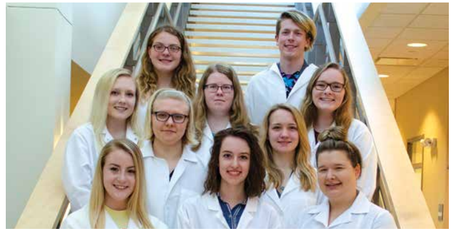




K-12

Scientists from The Hormel Institute participate in area science fairs to promote learning the scientific method and to award "The Hormel Institute OUTSTANDING Science Research Award" to students at various grade levels. Researchers also mentor young students by volunteering their time in the Science Fair Mentoring Project and working with students in the classroom to demonstrate techniques and talk about possible career paths. Every sixth grader in Austin gets an opportunity to tour The Hormel Institute, and as students advance in their studies, taking courses like honors biology, they have additional, increasingly impactful interactions with Institute researchers.

EDUCATION

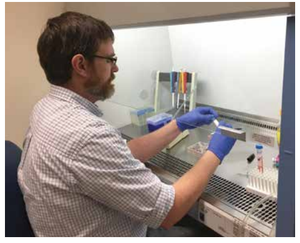


COLLEGE

The annual Summer Undergraduate Research Experience (SURE) internship program gives college undergraduate students the opportunity to work with The Hormel Institute scientists on research projects to expand their knowledge of basic research as well as to learn about equipment and techniques that generally are not available in undergraduate academic programs.

TOMORROW'S SCIENTISTS

The Hormel Institute has been part of University of Minnesota since its inception in 1942. Our mission is to conduct research and provide education in the biological sciences and share our knowledge with the world. The Hormel Institute serves as an education hub and center of technical and educational expertise for the benefit of the Austin community, surrounding region and State of Minnesota. Education is a core function and we strive to inspire interest in science and prepare people for successful biomedical careers.



POST-DOCs

The Hormel Institute is a hub for postdoctoral training and nearly every lab has a team that includes new PhDs. Postdoc is the de facto next step on the academic career path after earning a doctorate degree (Ph.D.). A postdoc is a temporary position that allows a PhD to continue his or her training as a researcher and gain necessary skills and experience to prepare them for the next steps in their research careers.

COMMUNITY

Educating students has been and remains an important part of the community outreach of The Hormel Institute, University of Minnesota. Our scientists and staff help inspire interest in science education throughout leadership in programs for both individuals and organizations, including:

- Advise/serve on dissertation committee for doctoral students
- BICB - graduate training for Biomedical Informatics and Computational Biology students at UMN-Rochester
- GRAUC (Greater Rochester Advocates for Universities and Colleges board) advocate for education in southern Minnesota
- Austin Aspires
- Riverland Community College - support "Be Your Best"
- Austin Science Fair Mentoring Project - mentor students

RESEARCH-FOCUSED

PUBLICATIONS

H.I. No. 2247 *Treatment implications of natural compounds targeting lipid metabolism in nonalcoholic fatty liver disease, obesity and cancer.* Cheng, C., Zhuo, S., Zhang, B., Zhao, X., Liu, Y., Liao, C., . . . Luo, X. Int J Biol Sci, *15*(8), 1654-1663. (2019).

H.I. No. 2248 *CELF2 suppresses non-small cell lung carcinoma growth by inhibiting the PREX2-PTEN interaction.* Yeung, Y. T., Fan, S., Lu, B., Yin, S., Yang, S., Nie, W., . . . Dong, Z. Carcinogenesis. (2019).

H.I. No. 2249 *A proteomic analysis of differentiating dopamine neurons derived from human embryonic stem cells.* Ryu, J., Park, B. C., & Lee, D. H. Anim Cells Syst (Seoul), *23*(3), 219-227. (2019).

H.I. No. 2250 *Lapachol is a novel ribosomal protein S6 kinase 2 inhibitor that suppresses growth and induces intrinsic apoptosis in esophageal squamous cell carcinoma cells.* Zu, X., Xie, X., Zhang, Y., Liu, K., Bode, A. M., Dong, Z., & Kim, D. J. Phytother Res. (2019).

H.I. No. 2251 *EBV(LMP1)-induced metabolic reprogramming inhibits necroptosis through the hypermethylation of the RIP3 promoter.* Shi, F., Zhou, M., Shang, L., Du, Q., Li, Y., Xie, L., . . . Cao, Y. Theranostics, *9*(9), 2424-2438. (2019).

H.I. No. 2252 *Inhibition of LTA4H by bestatin in human and mouse colorectal cancer.* Zhao, S., Yao, K., Li, D., Liu, K., Jin, G., Yan, M., . . . Dong, Z. EBioMedicine, *44*, 361-374. (2019).

H.I. No. 2253 *Structural basis of phosphatidylcholine recognition by the C2-domain of cytosolic phospholipase A2alpha.* Hirano, Y., Gao, Y. G., Stephenson, D. J., Vu, N. T., Malinina, L., Simanshu, D. K., . . . Brown, R. E. Elife, *8*. (2019).

H.I. No. 2254 *Structural variation of centromeric endogenous retroviruses in human populations and their impact on cutaneous T-cell lymphoma, Sezary syndrome, and HIV infection.* Kaplan, M. H., Kaminski, M., Estes, J. M., Gitlin, S. D., Zahn, J., Elder, J. T., . . . Contreras-Galindo, R. BMC Med Genomics, *12*(1), 58. (2019).

H.I. No. 2255 *Cell growth inhibition by 3-deoxysappanchalcone is mediated by directly targeting the TOPK signaling pathway in colon cancer.* Zhao, R., Huang, H., Choi, B. Y., Liu, X., Zhang, M., Zhou, S., . . . Lee, M. H. Phytomedicine, *61*, 152813. (2018).

H.I. No. 2256 *p300 Acetyltransferase Is a Cytoplasm-to-Nucleus Shuttle for SMAD2/3 and TAZ Nuclear Transport in Transforming Growth Factor beta-Stimulated Hepatic Stellate Cells.* Wang, Y., Tu, K., Liu, D., Guo, L., Chen, Y., Li, Q., . . . Kang, N. Hepatology. (2019).

H.I. No. 2257 *Nuclear factor-erythroid 2-related factor 3 (NRF3) is low expressed in colorectal cancer and its down-regulation promotes colorectal cancer malignance through activating EGFR and p38/MAPK.* Zhou, S., Li, Y., Lu, J., Chen, C., Wang, W., Wang, L., . . . Tang, F. Am J Cancer Res, *9*(3), 511-528. (2019).

H.I. No. 2258 *PGC1alpha/CEBPB/CPT1A axis promotes radiation resistance of nasopharyngeal carcinoma through activating fatty acid oxidation.* Du, Q., Tan, Z., Shi, F., Tang, M., Xie, L., Zhao, L., . . . Cao, Y. Cancer Sci, *110*(6), 2050-2062. (2019).

H.I. No. 2259 *Multifunctional Gold Nanoparticles Overcome MicroRNA Regulatory Network Mediated-Multidrug Resistant Leukemia.* Deng, R., Ji, B., Yu, H., Bao, W., Yang, Z., Yu, Y., . . . Yan, F. Sci Rep, *9*(1), 5348. (2019).

H.I. No. 2260 *The PGC-1/ERR network and its role in precision oncology.* De Vitto, H., Bode, A. M., & Dong, Z. NPJ Precis Oncol, *3*, 9. (2019).

H.I. No. 2261 *CRISPR/Cas9 - An evolving biological tool kit for cancer biology and oncology.* Tian, X., Gu, T., Patel, S., Bode, A. M., Lee, M. H., & Dong, Z. NPJ Precis Oncol, *3*, 8. (2019).

H.I. No. 2262 *ScanNeo: identifying indel derived neoantigens using RNA-Seq data.* Wang, T. Y., Wang, L., Alam, S. K., Hoeppner, L. H., & Yang, R. Bioinformatics. (2019).

H.I. No. 2263 *Posttranslational regulation of PGC-1 alpha and its implication in cancer metabolism.* Luo, X., Liao, C., Quan, J., Cheng, C., Zhao, X., Bode, A. M., & Cao, Y. Int J Cancer, *145*(6), 1475-1483. (2019).

H.I. No. 2264 *Modulation of calreticulin expression reveals a novel exosome-mediated mechanism of Z variant alpha1-antitrypsin disposal.* Khodayari, N., Oshins, R., Alli, A. A., Tuna, K. M., Holliday, L. S., Krotova, K., & Brantly, M. J Biol Chem, *294*(16), 6240-6252. (2019).

H.I. No. 2265 *AKT as a Therapeutic Target for Cancer.* Song, M., Bode, A. M., Dong, Z., & Lee, M. H. Cancer Res, *79*(6), 1019-1031. (2019).

H.I. No. 2266 *Aurora B kinase as a novel molecular target for inhibition the growth of osteosarcoma.* Zhao, Z., Jin, G., Yao, K., Liu, K., Liu, F., Chen, H., . . . Dong, Z. Mol Carcinog, *58*(6), 1056-1067. (2019).

H.I. No. 2267 *In Vitro Measurement of Sphingolipid Intermembrane Transport Illustrated by GLTP Superfamily Members.* Kenoth, R., Brown, R. E., & Kamlekar, R. K. Methods Mol Biol, *1949*, 237-256. (2019).

H.I. No. 2268 *3,3'-Diindolylmethane inhibits patient-derived xenograft colon tumor growth by targeting COX1/2 and ERK1/2.* Tian, X., Liu, K., Zu, X., Ma, F., Li, Z., Lee, M., . . . Kim, D. J. Cancer Lett, *448*, 20-30. (2019).

H.I. No. 2269 *Dimerization defective MODY mutations of hepatocyte nuclear factor 4alpha.* Singh, P., Tung, S. P., Han, E. H., Lee, I. K., & Chi, Y. I. Mutat Res, *814*, 1-6. (2019).

H.I. No. 2270 *Transforming growth factor beta (TGF-beta) cross-talk with the unfolded protein response is critical for hepatic stellate cell activation.* Liu, Z., Li, C., Kang, N., Malhi, H., Shah, V. H., & Maiers, J. L. J Biol Chem, *294*(9), 3137-3151. (2019).

H.I. No. 2271 *3-Iodothyronamine Acting through an Anti-Apoptotic Mechanism Is Neuroprotective Against Spinal Cord Injury in Rats.* Lv, J., Liao, J., Tan, W., Yang, L., Shi, X., Zhang, H., . . . Li, Q. Ann Clin Lab Sci, *48*(6), 736-742. (2018).

H.I. No. 2272 *Spontaneous Cancers, But Not Many Induced Ones in Animals, Resemble Semi-New Organisms that Possess a Unique Programmed Cell Death Mode Different from Apoptosis, Senescent Death, Necrosis and Stress-Induced Cell Death.* Shi, M., Zhou, H., Lei, M., Chen, L., Zellmer, L., He, Y., . . . Liao, D. J. J Cancer, *9*(24), 4726-4735. (2018).

H.I. No. 2273 *MICAL2 Mediates p53 Ubiquitin Degradation through Oxidating p53 Methionine 40 and 160 and Promotes Colorectal Cancer Malignance.* Lu, J., Li, Y., Wu, Y., Zhou, S., Duan, C., Dong, Z., . . . Tang, F. Theranostics, *8*(19), 5289-5306. (2018).

H.I. No. 2274 *Bone marrow-derived epithelial cells and hair follicle stem cells contribute to development of chronic cutaneous neoplasms.* Park, H., Lad, S., Boland, K., Johnson, K., Readio, N., Jin, G., . . . Morris, R. J. Nat Commun, *9*(1), 5293. (2018).

H.I. No. 2275 *Gossypin inhibits gastric cancer growth by direct targeting of AURKA and RSK2.* Wang, L., Wang, X., Chen, H., Zu, X., Ma, F., Liu, K., . . . Kim, D. J. Phytother Res, *33*(3), 640-650. (2019).

H.I. No. 2276 *Ethyl gallate as a novel ERK1/2 inhibitor suppresses patient-derived esophageal tumor growth.* Liu, F., Zu, X., Xie, X., Liu, K., Chen, H., Wang, T., . . . Kim, D. J. Mol Carcinog, *58*(4), 533-543. (2019).

H.I. No. 2277 *MNAT1 is overexpressed in colorectal cancer and mediates p53 ubiquitin-degradation to promote colorectal cancer malignance.* Zhou, S., Lu, J., Li, Y., Chen, C., Cai, Y., Tan, G., . . . Tang, F. J Exp Clin Cancer Res, *37*(1), 284. (2018).

H.I. No. 2278 *Upregulation of human glycolipid transfer protein (GLTP) induces necroptosis in colon carcinoma cells.* Mishra, S. K., Stephenson, D. J., Chalfant, C. E., & Brown, R. E. Biochim Biophys Acta Mol Cell Biol Lipids, *1864*(2), 158-167. (2019).

H.I. No. 2279 *Evaluating the Remote Control of Programmed Cell Death, with or without a Compensatory Cell Proliferation.* Dou, X., Chen, L., Lei, M., Zellmer, L., Jia, Q., Ling, P., . . . Liao, D. J. Int J Biol Sci, *14*(13), 1800-1812. (2018).

H.I. No. 2280 *Gossypetin is a novel MKK3 and MKK6 inhibitor that suppresses esophageal cancer growth in vitro and in vivo.* Xie, X., Liu, K., Liu, F., Chen, H., Wang, X., Zu, X., . . . Kim, D. J. Cancer Lett, *442*, 126-136. (2019).

H.I. No. 2281 *Heterozygous deletion of chromosome 17p renders prostate cancer vulnerable to inhibition of RNA polymerase II.* Li, Y., Liu, Y., Xu, H., Jiang, G., Van der Jeught, K., Fang, Y., . . . Lu, X. Nat Commun, *9*(1), 4394. (2018).

H.I. No. 2282 *Vasohibin 2 promotes malignant behaviors of pancreatic cancer cells by inducing epithelial-mesenchymal transition via Hedgehog signaling pathway.* Zhang, Y., Xue, X., Zhao, X., Qin, L., Shen, Y., Dou, H., . . . Yang, D. Q. Cancer Med, *7*(11), 5567-5576. (2018).

H.I. No. 2283 *Mitochondrial network structure homeostasis and cell death.* Xie, L. L., Shi, F., Tan, Z., Li, Y., Bode, A. M., & Cao, Y. Cancer Sci, *109*(12), 3686-3694. (2018).

H.I. No. 2284 *The Chemosensory Function of Primary Cilia Regulates Cholangiocyte Migration, Invasion, and Tumor Growth.* Mansini, A. P., Peixoto, E., Jin, S., Richard, S., & Gradilone, S. A. Hepatology, *69*(4), 1582-1598. (2019).

H.I. No. 2285 *A dynamic N(6)-methyladenosine methylome regulates intrinsic and acquired resistance to tyrosine kinase inhibitors.* Yan, F., Al-Kali, A., Zhang, Z., Liu, J., Pang, J., Zhao, N., . . . Liu, S. Cell Res, *28*(11), 1062-1076. (2018).

H.I. No. 2286 *Recent advances in precision oncology research.* Bode, A. M., & Dong, Z. NPJ Precis Oncol, *2*, 11. (2018).

H.I. No. 2287 *iTRAQ and PRM-based quantitative proteomics in T2DM-susceptible and -tolerant models of Bama mini-pig.* Yan, X., Wu, Y., Zhong, F., Jiang, Q., Zhou, T., Guo, Y., . . . Lan, G. Gene, *675*, 119-127. (2018).

H.I. No. 2288 *Dinitrosopiperazine-decreased PKP3 through upregulating miR-149 participates in nasopharyngeal carcinoma metastasis.* Li, Y., Ju, K., Wang, W., Liu, Z., Xie, H., Jiang, Y., . . . Tang, F. Mol Carcinog, *57*(12), 1763-1779. (2018).

H.I. No. 2289 *Wild-type IDH2 promotes the Warburg effect and tumor growth through HIF1alpha in lung cancer.* Li, J., He, Y., Tan, Z., Lu, J., Li, L., Song, X., . . . Cao, Y. Theranostics, *8*(15), 4050-4061. (2018).

H.I. No. 2290 *Circulating Adipose Fatty Acid Binding Protein Is a New Link Underlying Obesity-Associated Breast/Mammary Tumor Development.* Hao, J., Zhang, Y., Yan, X., Yan, F., Sun, Y., Zeng, J., . . . Li, B. Cell Metab, *28*(5), 689-705.e685. (2018).

H.I. No. 2291 *BMI1 Drives Metastasis of Prostate Cancer in Caucasian and African-American Men and Is A Potential Therapeutic Target: Hypothesis Tested in Race-specific Models.* Ganaie, A. A., Beigh, F. H., Astone, M., Ferrari, M. G., Maqbool, R., Umbreen, S., . . . Saleem, M. Clin Cancer Res, *24*(24), 6421-6432. (2018).

H.I. No. 2292 *An Adeno-Associated Viral Vector Capable of Penetrating the Mucus Barrier to Inhaled Gene Therapy.* Duncan, G. A., Kim, N., Colon-Cortes, Y., Rodriguez, J., Mazur, M., Birket, S. E., . . . Suk, J. S. Mol Ther Methods Clin Dev, *9*, 296-304. (2018).

H.I. No. 2293 *The Ashitaba (Angelica keiskei) Chalcones 4-hydroxyderricin and Xanthoangelol Suppress Melanomagenesis By Targeting BRAF and PI3K.* Zhang, T., Wang, Q., Fredimoses, M., Gao, G., Wang, K., Chen, H., . . . Dong, Z. Cancer Prev Res (Phila), *11*(10), 607-620. (2018).

H.I. No. 2294 *Targeting PRPK and TOPK for skin cancer prevention and therapy.* Roh, E., Lee, M. H., Zykova, T. A., Zhu, F., Nadas, J., Kim, H. G., . . . Dong, Z. Oncogene, *37*(42), 5633-5647. (2018).

H.I. No. 2295 *Dual-specificity phosphatase 18 modulates the SUMOylation and aggregation of Ataxin-1.* Ryu, J., & Lee, D. H. Biochem Biophys Res Commun, *502*(3), 389-396. (2018).

H.I. No. 2296 *Therapies based on targeting Epstein-Barr virus lytic replication for EBV-associated malignancies.* Li, H., Hu, J., Luo, X., Bode, A. M., Dong, Z., & Cao, Y. Cancer Sci, *109*(7), 2101-2108. (2018).

H.I. No. 2297 *Pharmacological TLR4 Antagonism Using Topical Resatorvid Blocks Solar UV-Induced Skin Tumorigenesis in SKH-1 Mice.* Blohm-Mangone, K., Burkett, N. B., Tahsin, S., Myrdal, P. B., Aodah, A., Ho, B., . . . Dickinson, S. E. Cancer Prev Res (Phila), *11*(5), 265-278. (2018).

H.I. No. 2298 *A Pan-ALDH1A Inhibitor Induces Necroptosis in Ovarian Cancer Stem-like Cells.* Chefetz, I., Grimley, E., Yang, K., Hong, L. D., Vinogradova, E. V., Suciu, R., . . . Buckanovich, R. J. Cell Reports, *26*(11), 3061-+. (2019).

H.I. No. 2299 *Structural analyses of 4-phosphate adaptor protein 2 yield mechanistic insights into sphingolipid recognition by the glycolipid transfer protein family.* Ochoa-Lizarralde, B., Gao, Y. G., Popov, A. N., Samygina, V. R., Zhai, X. H., Mishra, S. K., . . . Malinina, L. Journal of Biological Chemistry, *293*(43), 16709-16723. (2018).

H.I. No. 2300 *Epidermal FABP Prevents Chemical-Induced Skin Tumorigenesis by Regulation of TPA-Induced IFN/p53/SOX2 Pathway in Keratinocytes.* Zhang, Y. W., Hao, J. Q., Zeng, J., Li, Q., Rao, E. Y., Sun, Y. W., . . . Li, B. Journal of Investigative Dermatology, *138*(9), 1925-1934. (2018).

SEMINARS

July 10, 2018
Jyoti R. Misra, PhD
2nd Interview
Postdoctoral Associate
Waksman Institute of Microbiology
Rutgers University
"Novel Regulators of Growth Control"

July 17, 2018
Chang-Wei Liu, PhD
2nd Interview
Associate Professor
Department of Biochemistry and Molecular Genetics
University of Colorado School of Medicine
"Regulating Apoptosis by Deubiquitinase USP35"

July 24, 2018
Bhaskar Das, PhD
2nd Interview
Associate Professor
Departments of Medicine and Pharmacological Sciences
Icahn School of Medicine at Mount Sinai
"Development of Retinoid Based and Pharmacological Agents for Brain Development and Diseases"

August 7, 2018
Jiwang Zhang, MD, PhD
2nd Interview
Associate Professor
Department of Pathology
Loyola University Chicago
"Bone Marrow Niche Signaling in the Regulation of Normal and Disease Hematopoiesis"

August 14, 2018
Mudit Tyagi, PhD
2nd Interview
Assistant Professor
Department of Medicine
Department of Microbiology, Immunology, and Tropical Medicine
The George Washington University
"HIV latency, the major hurdle in HIV eradication; and the impact of cocaine on HIV gene expression and replication"

August 16, 2018
Stephen M. Ansell, MD, PhD
Professor of Medicine
Mayo Clinic
"Modulating Immune Function in Lymphoma"

August 17, 2018
Svetomir N. Markovic, MD, PhD
Professor of Medicine
Professor of Oncology
Mayo Clinic
"Melanoma Immunotherapy"

August 28, 2018
Amer Alam, PhD
2nd Interview
Scientific Coordinator
ETH Zurich
"Molecular insights into drug transport and inhibition in human ABCB1/p-glycoprotein"

August 30, 2018
Jake Hines, PhD
Assistant Professor
Winona State University
"The CNS myelin landscape: mechanisms of axon recognition and initial myelin sheath formation"

September 4, 2018
Xu Lou, PhD
2nd Interview
Associate Professor
Eppley Institute for Cancer Research
University of Nebraska Medical Center
"How do cells unlock the gateway to apoptosis?"

September 11, 2018
Zain Paroo, PhD
2nd Interview
Assistant Professor
Department of Pharmacology
University of Illinois, Chicago
"RNA metabolism in disease and drug discovery"

September 25, 2018
Liang Liu, PhD
2nd Interview
Assistant Professor
Department of Dermatology
Columbia University
"Interplay of Genetic and Epigenetic Factors in UV-induced Skin Tumorigenesis"

October 4, 2018
Colleen Meyers
Education Program Specialist
University of Minnesota
"English Pronunciation for Academics"

October 4, 2018
Elena Stetsenko
Education Program Specialist
University of Minnesota
"English Pronunciation for Academics"

October 9, 2018
Feng Zhang, PhD
2nd Interview
Assistant Professor
Department of Neurology
University of Pittsburgh
"Nrf2 in Ischemic Brain"

October 11, 2018
G.W. Gant Luxton, PhD
Assistant Professor
Genetics, Cell Biology, and Development
University of Minnesota
"Building and breaking nucleo-cytoskeletal bridges: LINC complexes in health and disease"

October 16, 2018
Rafael Contreras, PhD
2nd Interview
Research Assistant Professor
Department of Internal Medicine
Division of Infectious Diseases
University of Michigan
"Instability of Centromere Repeats and Retroelements in Fibrosis and Cancer"

October 19, 2018
Hon S. Leong, Msc, PhD
2nd Interview
Senior Associate Consultant II
Department of Urology
Mayo Clinic
"Translating Insights Gained from Intravital Imaging of Cancer Metastasis to Transform Cancer Patient Outcomes"

October 23, 2018
Wioletta Czaja, PhD
2nd Interview
Assistant Research Scientist
University of Georgia
"Finding the balance within: Integrating research on mechanisms of genome stability"

October 25, 2018
Justin Mott, MD, PhD
Associate Professor
Department of Biochemistry & Molecular Biology
University of Nebraska Medical Center
"FGFR4 Promotes Cholangiocarcinoma Progression"

November 8, 2018
Thorunn Bjarnadottir
Education Program Specialist
GPS Glpbal Programs & Strategy
University of Minnesota
"Getting to Know Your Own Culture"

November 16, 2018
Karen Kaehler
Senior Technology Portfolio Manager
Office of Technology Commercialization
University of Minnesota
"Updates from Office of Technology Commercialization"

November 16, 2018
Mary MacCarthy
Venture Center Program Manager
Office of Technology Commercialization
University of Minnesota
"Updates from Office of Technology Commercialization"

December 6, 2018
Scott H. Kaufmann, MD, PhD
Professor of Medicine
Professor of Pharmacology
Division of Oncology Research
Department of Oncology
Mayo Clinic
"A Research Seminar & Spore Grants"

December 13,2018
Masato Yamamoto, MD, PhD
Professor
Division of Basic & Translational Research
Department of Surgery
University of Minnesota
"TBD"

December 20, 2018
Jason Mears, PhD
Assistant Professor
Department of Pharmacology
Case Western Reserve University
"TBD"

January 10, 2019
Tim Starr, PhD
Assistant Professor
Department of Obstetrics, Gynecology and Women's Health (OBGYN)
University of Minnesota
Correlation of Single Cell RNA-Seq Data with Chemotherapy Response"

February 21, 2019
Amir Lerman, MD
Professor of Medicine
Department of Cardiovascular Medicine
Mayo Clinic
"Tissue engineering and regenerative medicine"

April 18, 2019
Jean-Pierre A. Kocher, PhD
Professor of Biomedical Informatics
Mayo Clinic
"Bioinformatics Core"

April 25, 2019
Gen-Sheng Feng, PhD
Professor of Pathology & Biology
UC San Diego
"Deciphering the anti-oncogenic effects of classical oncoproteins in liver cancer"

May 2, 2019
Federico Rey, PhD
Assistant Professor of Bacteriology
University of Wisconsin – Madison
"Gut bacterial metabolism and human health"

May 9, 2019
Kenneth Beckman, PhD
Director
UMN Genomics Center
"Alternative Careers after PhD"

June 4, 2019
James M. Daley, PhD
Associate Research Scientist
Dept. of Molecular Biophysics & Biochemistry
Yale University School of Medicine
"BRCA1-BARD1 promotes end resection of DNA double-strand breaks"

June 6, 2019
Hui Xu, PhD
Professor
Department of Dermatology
University of Alabama, Birmingham
"Senescence associated secretory phenotype in ultraviolet radiation induced immune suppression"

June 25, 2019
Jarrod B. French, PhD
Assistant Professor
Dept. of Chemistry
Dept. of Biochemistry & Cell Biology
Stony Brook University
Optimizing the immune system to fight disease; and A 'complex' way to regulate nucleotide metabolism"

INCOME

Income from Grants, Contracts and Development

National Institutes of Health

National Cancer Institute
- Chemoprevention of Colon Cancer by Targeting the Wnt/beta Catenin Pathway (Z. Dong) $240,760
- Prevention of solar UV-induced Skin Cancer by Targeting LTZ4H (Z. Dong) $222,552
- Developing New Ornithine Decarboxylase Inhibitors to Prevent Skin Cancer (Z. Dong) $93,375
- Hepatic Stellate Cell Regulation of Metastatic Growth in the Liver (N. Kang) $213,750
- Targeting High Fat Diet-Driven DNA hypermethylation for AML chemoprevention (S. Liu) $50,000
- The Cholangiocyte Primary Cilium as a Tumor Suppressor Organelle (S. Gradilone) $228,750

National Institute of Health
- Targeting eIF4A1-dependent HK2 translation axis for prevention of castrationresistant prostate cancer (Y. Deng) $266,754
- Mechanism of high-efficiency transduction of hepatocytes by optimized AAV vectors (G. Aslanidi) $86,744

American Cancer Society
- Determination & validation of resistance mechanisms in melanoma (J. Robinson) $156,157
- Institutional Research Grant (L. Hoeppner) $30,000

Foundation for Women's Cancer
- Necroptosis in ovarian cancer cells with gain of function of p53 mutations (I. Chefetz-Menaker) $54,534

Life Science Research Foundation
- Identification and characterization of cancer stem cells in ovarian cancer (I. Chefetz-Menaker) $12,936

Ovarian Center Research Fund
- Drugs Inducing Necroptotic Cell Death as a New Startegy for high grade ovarian cancer (I. Chefetz-Menaker) $150,000

Pardee Foundation
- Necroptosis: molecular and metabolic aspects (I. Chefetz-Menaker) $54,534

Pennsylvania State University
- Prevention of prostate carcinogenesis by next-generation selenium (Y. Deng) $46,374
- Translational development studies of a novel combination therapy for castration-resistant prostate cancer (Y.Deng) $80,000

Prostate Cancer Foundation
- Delineating coding and regulatory indels in prostate cancer (R. Yang) $75,000

University of Florida/NIH
- Mechanism of high-efficiency transduction of hepatocytes by optimized AAV vectors (G. Aslanidi) $118,186

University of Minnesota Medicinal Chemistry/NIH
- Smoking-induced epigenetic changes in the lung: Role of DNA demethylation (S. Liu) $97,953

University of South Florida/NIH
- The Functional Role of the cPLA2alpha/C1P Interaction in Sepsis Resolution (E. Hinchcliffe) $123,047
- The Functional Role of the cPLA2alpha/C1P Interaction in Sepsis Resolution (R. Brown) $142,633

Department of Defense
- Cellular mechanisms underlying pediatric glioblastoma: Heterozygous mutations in histone H3.3 induce chromosome instability by abolishing Ser31 phosphorylation (T. Hinchliffe) $166,667

Mayo Clinic Rochester
- Mayo Clinic - Hormel Institute collaboration (S. Liu) $43,000

PhRMA Foundation
- Accurate detection of structrual variation from DNA sequencing of circulatinf tumor cells (R. Yang) $63,158

Bob Allison Ataxia Research Center
- Function of ATM in Neuronal Survival in Response to Growth Factor Signaling (D. Yang) $15,000

University of Minnesota Foundation
- Discovery of a novel beta-catenin inhibitor that shows potent therapeutic effects against colone cancer (S. Shin) $62,000

Prevent Cancer Foundation
- A novel UV signature for assessing skin UV damage and cancer risk (L. Liu) $50,000

Other Resources

The Hormel Foundation	$4,538,500
University of Minnesota	$400,000
Indirect Cost Return	$887,753
Fundraising/Development	$1,123,822

Total **$9,893,939**

ADVISORY BOARD

The Hormel Institute Advisory Board

Ann M. Bode, Ph.D.
Interim Executive Director,
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Rochester, MN

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The research, partnerships and resources of
The Hormel Institute are dedicated to a single purpose:
Improving health through medical research.

Accelerating **ANSWERS**
to **CANCER**