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2013 - 2014 ANNUAL REPORT

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THE HORMEL INSTITUTE
UNIVERSITY OF MINNESOTA

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

Today's RESEARCH,
Tomorrow's CURES

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Today's RESEARCH, Tomorrow's CURES

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 DIRECTOR
DR. ZIGANG DONG

Cancer affects all of humankind: women and men, poor and rich, old and young, and all races. Cancer is the leading cause of death worldwide. Most human cancers are preventable or treatable. History has proven that only research will lead to new methods for prevention and therapy of human cancer. The Hormel Institute is a leading medical research institute making major contributions to the identification and characterization of novel molecular and cellular targets and agents for cancer prevention and therapy. During 2013-14, The Hormel Institute experienced continued success obtaining research funding and producing major research breakthroughs, even in a national environment of overall decreased funding for research.

With the support of State Senator Dan Sparks and State Representative Jeanne Poppe, as well as State Senator David Senjem, the State of Minnesota bonding bill, signed by Governor Mark Dayton, included $13.5 million in funding to the City of Austin Port Authority to lead the construction project for The Hormel Institute's next expansion. The Hormel Foundation, led by Chairman Gary Ray, announced $23 million in funding to support the project. The construction is currently in the design process and groundbreaking was held on May 28, 2014. The new building will be home to the International Center for Research Technology (ICRT) and The Hormel Institute.

The ICRT is a collaborative project with manufacturers of technology, such as IBM Rochester, to develop new technology to accelerate discoveries and facilitate comprehensive study of human diseases by combining analysis of protein structure/function with advanced methods of data management and drug screening. U.S. Congressman Tim Walz and U.S. Senators Al Franken and Amy Klobuchar have shown strong support to The Hormel Institute by acquiring funding for the purchase of high-end equipment to establish the ICRT.

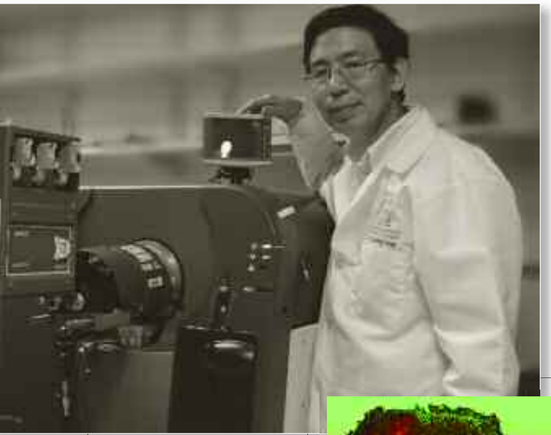
We are thankful for the generous support of The Hormel Foundation, Hormel Foods Corporation, University of Minnesota, and Mayo Clinic. In particular, I would like to thank Mr. Gary Ray, Mr. Jeff Ettinger, Mr. Richard Knowlton, Mr. Joel Johnson, Mrs. Bonnie Rietz, Mr. Jerry Anfinson, and Mr. Steve Rizzi. We thank Drs. Eric Kaler and Brian Herman (University of Minnesota) and Drs. John Noseworthy, Glenn Forbes, Robert Diaiso, and Greg Gores (Mayo Clinic) for their leadership and support. We thank our elected leaders, Minnesota Governor Mark Dayton, U.S. Senators Al Franken and Amy Klobuchar, U.S. Representative Tim Walz, Minnesota State Senator Dan Sparks, Minnesota State Representative Jeanne Poppe, State Senator David Senjem, and Mayor Tom Stiehm for their continued support. We remain deeply grateful to our community, our partners and our collaborators for giving us the gift of allowing us to work here. Their support and gifts allow today's research to flourish and pave the way for tomorrow's progress to continue.

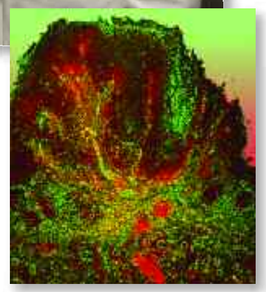
"Most human cancers are preventable, or treatable, if discovered at an early stage."

Dr. Zigang Dong
Executive Director

CELLULAR AND MOLECULAR BIOLOGY

Executive Director/Section Leader
Zigang Dong, M.D., Dr. P.H.
McKnight Presidential Professor in Cancer Prevention
Hormel/Knowlton Professor





Many proteins are overexpressed only in cancer. The epidermal growth factor (green) is highly expressed in skin tumors and is a major chemotherapy target in breast cancer.

Cancer is one of the leading causes of human death worldwide. Throughout history, humankind has won the battle against deadly diseases, including small pox and polio, by defeating them through prevention and treatment. By focusing on the molecular mechanisms explaining how normal cells can undergo neoplastic transformation induced by carcinogens and tumor promoters, we have discovered that key protein kinases, transcription factors, and other signaling molecules are critical factors in cancer development and are significant targets for cancer prevention and treatment.

1. The discovery that signal transduction pathways induced by UVA, UVB and solar UV are major etiological causative factors for human skin cancer and other diseases. We were the first to elucidate key signals/proteins that play functional roles in human carcinogenesis, especially solar ultraviolet (SUV)-, UVA- and UVB-induced skin cancers. Skin cancer is the most common cancer in the United States. The total incidence of all other cancers in the Americas is almost equal to the number of skin cancers, including melanoma and non-melanoma skin cancer. Because UVC is blocked by the ozone layer, UVA and UVB are the main components of solar UV that can reach the surface of the earth and penetrate human skin. We were the first group to systematically elucidate these signal transduction pathways in skin carcinogenesis. These signaling proteins include protein kinase C, extracellular signal-regulated kinases (ERKs), c-Jun N-terminal kinases (JNKs), ribosomal S6 kinase (RSK), mitogen- and stress-activated protein kinase (MSK), lymphokine-activated killer T-cell-originated protein kinase (TOPK), and transcription factors activator protein-1 (AP-1), nuclear factor kappa B (NF-kappa B), nuclear factor of activated T cells (NFAT), p53, and others. Notably, we showed that UV irradiation directly activates cannabinoid receptors 1 and 2 (CB1/2). Particularly, our data indicated that the absence of the CB1/2 receptors in mice results in a dramatic resistance to UVB-induced inflammation and a marked decrease in UVB-induced skin carcinogenesis. We also reported that the p38-related signal transduction pathway induced by SUV irradiation is critical for SUV-induced skin carcinogenesis. We also have tested the effects of many natural compounds in the prevention of SUV-induced skin cancer and identified their molecular targets. Taxifolin (a flavanonol found in macai palm, silymarin and red onion), for example, reportedly exerts multiple biologic effects, and we studied the molecular mechanisms and direct target(s) of taxifolin in skin cancer chemoprevention.

2. The discovery that protein kinases and their target transcription factors, including activator protein-1 (AP-1) are critical in cancer development and significant targets for cancer prevention. My group has studied AP-1 for many years and published almost 50 papers focusing on its mechanism of action and the effectiveness of preventing its activation in cancer cells. We were one of the first groups to propose transcriptional regulation as a target for cancer prevention. Our results provided the first in vivo evidence that the antitumor effects of retinoids are mediated by blocking AP-1 activity, but not by activation of RARE. We reported a novel function of TRAF2 in the epidermal growth factor (EGF) signaling pathway. RSK2, a member of the p90RSK family of proteins, is a widely expressed serine/threonine kinase that is activated by ERK1/2 and phosphoinositide-dependent kinase 1 in response to many growth factors and peptide hormones. Its activated signaling enhances cell survival. We resolved the X-ray structure of the RSK2 C-terminal kinase domain at 2.0-A resolution and discovered a C-terminal autoinhibitory alphaL-helix that is embedded in the kinase scaffold and determines the inactive kinase conformation. We also found that TOPK, a member of the MAPKK protein family, is highly expressed in human colorectal cancer tissues and cell lines and plays an important role in the transformation of colorectal cancer.

> "We discovered critical factors in cancer development and significant targets for cancer prevention and treatment."
>
> *Dr. Zigang Dong*

3. The discovery that natural compounds, found in (e.g. food), modulate crucial cellular signal transduction pathways in cancer development and prevention. We were the first group to identify molecular targets/mechanisms of chemoprevention of human cancer. Many chemicals/agents isolated from fruit, vegetables, and traditional medicinal herbs have been shown to be effective chemopreventive agents in multiple animal models. Little was known, however, regarding their mechanism(s) of action or their molecular targets. We were the first group to systematically identify and evaluate the molecular targets of tea polyphenols and other phytochemicals from fruit, vegetables and herbs. Our research efforts resulted in the discovery of high-affinity molecular targets of the tea polyphenol, epigallocatechin gallate (EGCG). We reported the X-ray crystal structure of the Pin1/EGCG complex resolved at 1.9 A resolution. The human peptidyl prolyl cis/trans isomerase (Pin1) plays a critical role in oncogenic signaling. This article received high praise in a separate commentary, which included the observation that the work "set a new bar for the future study of natural products with chemopreventive activity." We first reported that RSK2 plays a critical role in proliferation and cell transformation induced by tumor promoters. These results were published as a cover story for *Cancer Research*. Norathyriol is a metabolite of mangiferin found in mango and is known to have anticancer activity. Mechanistic investigations determined that norathyriol acted as a direct inhibitor of ERK1/2 activity to attenuate UVB-induced phosphorylation in the mitogen-activated protein kinase signaling cascades. We confirmed the direct and specific binding of norathyriol with ERK2 through a co-crystal structural analysis.

(Left to right) Front row: Ann M. Bode and Zigang Dong

Middle row: Do Young Lim, Kun Yeong Lee, Qiushi Wang, Margarita Malakhova, Yuqiao Sheng, Tatyana Zykova, Jihye Kim, Seung Ho Shin, Mi Hee Park, Hiroyuki Yamamoto, Eunmiri Roh, Srinivasa Reddy, Kanamata Reddy

Back row: Cheng Juan Zhang, Chengcheng Shi, Yi Zhang, Sung-Young Lee, Joohyun Ryu, DongHoon Yu, Tianshun Zhang, Yan Li, Alyssa Langfald, Hong-Gyum Kim, Kibeom Bae, Tae-Gyu Lim

Not pictured: Kang Dong Liu, Cong Peng, Ke Yao, Naomi Oi, Wei-Ya Ma, Haitao Li, Hanyong Chen, Yang Fu, HyoSun Kim, Ge Yang, JongEun Kim, Wei Li

Our work was highly recognized on the National Cancer Institute (NCI) website. "Dr. Dong and his group have, therefore, evaluated the potential of tea polyphenols and other distinct dietary factors to prevent cancer due to their ability to effectively alter molecular targets within cancer cells. This research, focusing on cell signaling, identified tea polyphenol EGCG 'receptors' – high-affinity cellular proteins that bind to EGCG. In collaboration with scientists at IBM, Dr. Dong's group used the world's fastest super-computer, the Blue Gene/L, to establish a docking method for EGCG-targeting proteins. With this method, they found that insulin-like growth factor-1 receptor can directly bind EGCG. Identification of this cellular receptor represents the first, most critical step in understanding molecular and biochemical mechanisms of tea polyphenols' anticancer effect. Such studies have also inspired a powerful new technology for studying mechanisms and molecular targets of other cancer preventive agents." (www.cancer.gov/researchandfunding/MERIT/Dong)

4. The discovery that histones – proteins with a critical role in cell division – are regulated at different phosphorylation or regulatory sites in the cell by enzymes known as protein kinases. The nucleosome is the basic unit of chromatin and generally consists of approximately two turns of DNA wrapped around an octamer of core histone proteins. We have discovered that many protein kinases phosphorylate histone to play critical roles in cell mitosis, cell transformation and apoptosis. We reported a function of H2AX in cellular apoptosis. Our data indicated that H2AX phosphorylation is required for DNA ladder formation, but not for the activation of caspase-3; and the JNKs/H2AX pathway cooperates with the caspase-3/CAD pathway resulting in cellular apoptosis. We also reported the novel role of the complex of RSK2/p53/histone H3. The RSK2/p53/histone H3 complex contributes to chromatin remodeling and cell cycle regulation. We also further elucidated the role of histone H2AX phosphorylation in malignant transformation and cancer development and found that RSK2 directly phosphorylates histone H2AX at Ser139 and also at a newly discovered site, Ser16. The RSK2/H2AX signaling pathway negatively regulates the RSK2/histone H3 pathway and, therefore, maintains normal cell proliferation.

5. The discovery that embryonic stem cell self-renewal and differentiation are regulated by protein kinases. Somatic cells can be reprogrammed into induced pluripotent stem cells (iPSCs) by transduction of reprogramming factors, including Oct4, Sox2, Klf4, and c-Myc. A coordinated network of these factors presumably confer a pluripotency of iPSCs. We provided new evidence showing that a protein kinase, Akt, directly interacts with Sox2 and promotes its stabilization through phosphorylation at Thr118, which enhanced the transcriptional activity of Sox2 in embryonic stem cells (ESCs). We found that mitogen-activated protein kinases are directly involved in this "cellular switch." Our results demonstrated that JNK1 and 2 play a negative role in reprogramming to pluripotent stem cells by suppressing Klf4 activity. We found that ERK1 or ERK2 binds to the activation domain of Klf4 and directly phosphorylates Klf4 at Ser123. Our data provided a molecular basis for the role of ERK1 and ERK2 in regulating Klf4-mediated mouse ESC self-renewal. Nanog also is known to regulate human and mouse ESC self-renewal activity, and we further found that ERK1 also binds and phosphorylates Nanog. We demonstrated that ERKs-mediated Nanog phosphorylation plays an important role in self-renewal of ESCs through FBXW8-mediated Nanog protein stability.

6. Computational biology directed molecular biology study in cancer research. Working with IBM's Blue Gene supercomputer team, my group has developed cutting-edge methods of computational biology to study protein targets for cancer prevention and therapy. Our group was the first to use such methods to study protein-protein interactions and find new mechanisms of tumor suppressor p16 (Choi, B.Y., Choi, H.S., Ko, K., Cho, Y.Y., Zhu, F., Kang, B.S., Ermakova, S.P., Ma, W.Y., Bode, A.M. and Dong, Z. (2005). The tumor suppressor p16(INK4a) prevents cell transformation through inhibition of c-Jun phosphorylation and AP-1 activity. *Nat Struct Mol Biol*, 12, 699-707).

In 2006, we reported novel mechanisms for modifications of histone H2AX and their role in apoptosis. The manuscript was published in *Molecular Cell* (Lu, C., Zhu, F., Cho, Y.Y., Tang, F., Zykova, T., Ma, W.Y., Bode, A.M. and Dong, Z. (2006) Cell apoptosis: requirement of H2AX in DNA ladder formation, but not for the activation of caspase-3. Mol Cell, 23, 121-32). In the subsequent issue of *Molecular Cell*, Dr. Roger Davis wrote a commentary highlighting the significance of this discovery and stated that the study "extends our understanding of the role of JNK in apoptosis signaling. Previous studies have demonstrated that JNK can contribute to the activation of capsases. The study by Lu et al (2006) demonstrated that JNK may also contribute to the apoptotic response of cells to activated capsases by phosphorylating H2AX at a noncanonical site that is required for apoptotic DNA fragmentation. In addition, Lu et al (2006) demonstrated that H2AX is a site of signal integration between the JNK signaling pathway and the PIKK group of protein kinases, including ATM, ATR, and DNA-PK." Using this method, our group discovered novel drugs for cancer therapy and novel use of FDA-approved drugs for new uses, such as anti-cancer, anti-inflammation and other diseases.

7. Secondary modification of p53 and its role in carcinogenesis. Our group was the first to identify protein kinases, including p38, ERKs, JNKs, Fyn, MLTK and RSK as p53 protein kinases that phosphorylate p53 at different amino acid residues. We also investigated the role of such modifications in cell apoptosis, cell cycle, and tumorigenesis in detail. We have summarized our work in an invited review article published in *Nature Reviews Cancer* entitled, "Post-translational modification of p53 in tumorigenesis." The manuscript was identified as the "most-cited article in the field of Molecular Biology and Genetics during 2004-09" by Thomson Reuters *ScienceWatch*.

8. Novel anticancer drug discovery for chemotherapy and chemoprevention for lung, colon and skin cancers. A) Inhibitors for lung carcinogenesis and lung cancer. Non-small cell lung cancer (NSCLC) is the leading cause of cancer mortality worldwide. Despite progress in developing chemotherapeutics for the treatment of NSCLC, primary and secondary resistance limits therapeutic success. NSCLC cells exhibit multiple mutations in the epidermal growth factor receptor (EGFR), which cause aberrant activation of diverse cell signaling pathways. Suppression of the inappropriate amplification of EGFR downstream signaling cascades, therefore, is considered to be a rational therapeutic and preventive strategy for the management of NSCLC. Our initial molecular target–oriented virtual screening revealed that the ginger components, including [6]-shogaol; [6]-paradol; [6]-gingerol; and butein, a USP8 inhibitor, and 3,6,2′,4′,5′-pentahydroxy-flavone seem to be potential candidates for the prevention and treatment of NSCLC. Among the compounds, [6]-shogaol showed the greatest inhibitory effects against NSCLC cell proliferation and anchorage-independent growth. [6]-shogaol induced cell cycle arrest (G1 or G2/M) and apoptosis. Furthermore, [6]-shogaol inhibited Akt kinase activity, a downstream mediator of EGFR signaling, by binding with an allosteric site of Akt. Other inhibitors such as butein, a USP8 inhibitor and 3,6,2′,4′,5′-pentahydroxy-flavone, all showed potent inhibitory effects against lung cancer cells in vitro and in vivo. These inhibitors can overcome EGFR inhibitor resistance in lung cancer.

B) Inhibitors targeting colon carcinogenesis and colon cancer. The mitogen-activated protein kinase kinase 1 and 2 signaling pathway is a major component of the RAS (rat sarcoma)/RAF (rapidly accelerated fibrosarcoma)/MEK (mitogen-activated protein kinase kinase)/ERKs (extracellular signal-regulated kinases) signaling axis that regulates tumorigenesis and cancer cell growth. MEK is frequently activated in various cancers that have mutations in the KRAS and BRAF oncogenes. Therefore, the MEK and Wnt pathways have been suggested as therapeutic targets for inhibitor development against tumorigenesis. We have discovered three novel MEK inhibitors, referred to as CInQ-01, CInQ-03 and CInQ-06 and the novel β-catenin inhibitor, esculetin. All four inhibitors were highly effective in suppressing MEK1 and MEK2 in vitro kinase activity as well as anchorage-dependent and anchorage-independent cell growth. The inhibitory activity was associated with markedly reduced phosphorylation of ERKs and ribosomal S6 kinases. Furthermore, administration of CInQ-03 and esculetin inhibited colon cancer cell growth in an in vivo xenograft mouse model and showed no skin toxicity. Overall, these results suggest that these novel MEK and β-catenin inhibitors might be used for chemotherapy or prevention.

C) Inhibitors targeting skin carcinogenesis and skin cancer. Solar UV (SUV) irradiation is a major factor in skin carcinogenesis, the most common form of cancer in the United States. The MAPK cascades are activated by SUV irradiation. We found that p38 signaling is critical for skin carcinogenesis. The 90 kDa ribosomal S6 kinase (RSK) and mitogen and stress-activated protein kinase (MSK) proteins constitute a family of protein kinases that mediate signal transduction downstream of the MAPK cascades. In this study, phosphorylation of RSK and MSK1 was up-regulated in human squamous cell carcinoma (SCC) and SUV-treated mouse skin. Kaempferol, a natural flavonol found in tea, broccoli, grapes, apples, and other plant sources, is known to have anticancer activity, but its mechanisms and direct target(s) in cancer chemoprevention are unclear. Kinase array results revealed that kaempferol inhibited RSK2 and MSK1. Pull-down assay results, ATP competition, and in vitro kinase assay data revealed that kaempferol interacts with RSK2 and MSK1 at the ATP-binding pocket and inhibits their respective kinase activities. Mechanistic investigations showed that kaempferol suppresses RSK2 and MSK1 kinase activities to attenuate SUV-induced phosphorylation of cAMP-responsive element binding protein (CREB) and histone H3 in mouse skin cells. Kaempferol was a potent inhibitor of SUV-induced mouse skin carcinogenesis. Further analysis showed that skin from the kaempferol-treated group exhibited a substantial reduction in SUV-induced phosphorylation of CREB, c-Fos, and histone H3. Overall, our results identify kaempferol as a safe and novel chemopreventive agent against SUV-induced skin carcinogenesis that acts by targeting RSK2 and MSK1.

Chrysin (5,7-dihydroxyflavone), a natural flavonoid widely distributed in plants, reportedly has chemopreventive properties against various cancers. The anticancer activity of chrysin observed in in vivo studies, however, has been disappointing. A chrysin derivative, referred to as compound 69407, more strongly inhibited EGF-induced neoplastic transformation of JB6 P+ cells compared with chrysin. It attenuated cell cycle progression of EGF-stimulated cells at the G1 phase and inhibited the G1/S transition. Compound 69407 reduced tumor growth in the A431 mouse xenograft model and retinoblastoma phosphorylation at Ser-795 and Ser-807/811. Immunoprecipitation kinase assay results showed that compound 69407 attenuated endogenous Cdk4 and Cdk2 kinase activities in EGF-stimulated JB6 P+ cells. Overall results indicated that compound 69407 is an ATP-noncompetitive cyclin-dependent kinase inhibitor with anti-tumor effects, which acts by binding inside the Cdk2 allosteric pocket. This study provides new insights for creating a general pharmacophore model to design and develop novel ATP-noncompetitive agents with chemopreventive or chemotherapeutic potency.

MOLECULAR CHEMOPREVENTION AND THERAPEUTICS

Section Leader

Mohammad Saleem Bhat, Ph.D.

Assistant Professor

24 weeks, Transgenic adenocarcinoma of the mouse prostate (TRAMP)

The long-term goals of this section are the following:

1. Understanding the biochemical, cellular and molecular processes that are crucial for the development of hormone-related (prostate and breast cancer) and lethal (pancreatic and colon cancer) cancers.
2. Identifying potential agents that could be used to treat and prevent cancer in humans.
3. Identifying novel tissue, serum and urine-based diagnostic and predictive biomarkers for prostate and breast cancer.
4. Understanding the causes of disparity in prostate and breast cancer diagnosis and outcome of therapy in African-Americans.

The major focus of our laboratory is in the area of translational research. The following programs are underway in our laboratory:

Research projects underway

1. Investigation of mechanisms of chemoresistance in prostate cancer patients

Prostate cancer is the most-common visceral cancer diagnosed in men. It is the second-leading cause of cancer-related deaths in males in the United States and the Western world. The lack of effective therapies for advanced prostate cancer reflects, to a large extent, the paucity of knowledge about the molecular pathways involved in prostate cancer development. After undergoing chemotherapy and radiotherapy, several cancer patients come back to the clinics with recurrence of aggressive forms of the disease. Thus, the identification of new predictive biomarkers will be important for improving clinical management, leading to improved survival of patients with prostate cancer. Such molecular targets, especially those that are indicative of proliferation, invasiveness of the disease and survival of cancerous cells (even after chemotherapy) also will be excellent candidate targets for staging the disease and establishing effectiveness of therapeutic and chemopreventive intervention of prostate cancer. We investigate the molecular mechanism that causes the failure of chemotherapy and radiotherapy in cancer patients. We have identified several molecules (genes and gene products) responsible for the development and recurrence of aggressive forms of cancer. These include S100A4 (a calcium-binding protein), BMI-1 (a polycomb group gene and stem cell factor), cFLIP (a casapse-8 inhibitor) and matriptase (a serine protease). The main objective of these studies is to take the bench-side research to the bed-side use in clinics.

2. Role of cancer stem cells in prostate cancer development and outcome of therapy

The critical pathological processes that occur during the development and progression of human prostate cancer and are known to confer aggressiveness to cancer cells are (1) abolishment of senescence of normal prostate epithelial cells, (2) self-renewability of prostate cancer cells even after chemotherapy and radiation, and (3) dysregulated cell cycle resulting in unchecked proliferation of cancer cells. Cellular senescence is physiologically important because it is a potent tumor suppressor mechanism that must be overcome for cells to be immortalized and transformed. Self-renewability of tumor cells is an essential defining property of a pluripotent stem cell–like phenotype of cancer cells that distinguishes it from other cell types. Stem cell–resembling population of cancer cells among the heterogeneous mix of cells constituting a tumor have been reported to be essential for tumor progression and metastasis of epithelial malignancies. The data generated from our laboratory suggest that several cancer cells that do not respond to chemotherapy or radiotherapy possess the traits of stem cells, thus, regenerating themselves even after chemo or radiotherapy treatment. The polycomb group (PcG) family of proteins (which form multimeric, gene-repressing complexes) has been reported to be involved in self renewability, cell cycle regulation, and senescence. BMI-1 is a transcription repressor and has emerged as an important member of the PcG family. We are investigating the role for BMI-1 protein in prostate cancer development. We hypothesize that the BMI-1 protein could be developed as a diagnostic and prognostic of prostate cancer.

> "Identification of new predictive biomarkers will be important for improving clinical management, leading to improved survival of patients with prostate cancer.
>
> *Dr. Mohammad Saleem Bhat*

3. Reactivation of Tumor Suppressor Genes

Early development of cancer is largely dependent upon androgens, and simultaneous suppression of tumor-suppressor genes predispose the initiated and premalignant prostate epithelial cells to acquire malignant phenotype. Among the phenotypic changes, the premalignant cells acquire increased motility, changes in cytoskeleton, changes in cell adhesion characteristics, and increased tendency for clonal expansion. The interaction between SLIT-ligand and its receptor Roundabout (Robo-1) is reported to guide axons during development of the nervous system. During organogenesis, the SLIT–ROBO pathway regulates numerous processes, including cell proliferation, migration and adhesion that seem to be important in the development of disparate tissues, including those of the reproductive system. SLIT-ROBO1 signaling has been shown to promote cell adhesion by stimulating the interaction between E-cadherin and beta-catenin at the plasma membrane. Various studies suggest the SLIT/ROBO network acts as a tumor-suppressor system in humans. We have started a broad program that is aimed at delineating the mechanism of action (tumor-suppressor action) of ROBO in human cancers. We are investigating whether reactivation of the ROBO system (in cancer cells within tumors) would stop the proliferation and dissemination of tumor cells to other body organs.

To test our hypothesis, we are adopting novel approaches, such as combining gene therapy and chemotherapy. Our focus currently is to test our hypothesis in prostate, pancreatic and skin (melanoma) cancers. We are running this program in collaboration with the Division of Translation Studies at the University of Minnesota's Masonic Cancer Center. This program has high translational potential for cancer patients.

(Left to right) Front row: Neelofar Jan Bhat, Mohammad Saleem Bhat, Aeijaz Sultan Parray
Back row: Kelly Gray (intern), Firdous Beigh, Michael Brickman (intern)

4. Role of S100A4 in the development of prostate cancer

S100A4, also known as mts1, CAPL, p9Ka, and metastasin, belongs to the S100 super-family of calcium-binding proteins and is located in a 2.05 Mbp segment of the genomic DNA of chromosome 1q21 region, where most of the S100 family of gene cluster occurs. S100A4 protein has been reported to be associated with the invasion and metastasis of cancer cells as well as frequently over-expressed in

metastatic tumors; normal cells with uninhibited movement, such as macrophages; transformed cells; and in various cancer types, such as breast, ovary, thyroid, lung, esophageal squamous cell carcinoma, gastric, colon, and prostate. Earlier, we reported that S100A4 is overexpressed during progression of prostate cancer in humans and TRAMP mice, an autochthonous transgenic model that develops prostate cancer in a manner similar to human disease. We recently showed that S100A4 regulates the events leading to proliferation and invasion of prostate cancer cells. We showed that S100A4 guides the invasive phenomenon of prostate cancer cells by regulating transcription and function of matrix metalloproteinase (MMP-9) in prostate cancer cells. S100A4 is notably known for its role in metastasis. By creating a transgenic mouse model of prostate cancer lacking S100A4, we – for the first time – provide evidence that the S100A4 protein, both in its intracellular and extracellular form, plays a tumor-promoting role in the development of prostate cancer by regulating the function of Nuclear Factor kappa B/Receptor for Advanced Glycation End products molecular circuitry.

5. Transition of androgen-depedent prostate cancer to androgen-independent phenotype

Aberrant Androgen receptor (AR) expression and activation promoted by mutations and binding partner mis-regulation is presented in several clinical manifestations, including androgen insensitivity syndrome, acne vulgaris, androgenetic alopecia, benign prostate hyperplasia (BPH), and different types of cancers in humans. AR has been found to be a principal driver of initiation and progression of prostate cancer. The initial stage of prostate cancer is dependent on androgen and can be managed by a series of therapies that are antagonist to AR or suppress AR signaling. The success of these therapies, however, is temporary, and, after a short remission period, tumors reappear as androgen-independent or commonly known as castration-resistant prostate cancer (CRPC). It is noteworthy that FDA-approved agents (androgen receptor signaling inhibitors), such as Bicalutamide, that are widely used in clinics to treat cancer show dismal results in men with advanced prostatic malignancy. It recently has been observed that overexpression of AR is the most-common event associated with CRPC. AR (which generally responds to androgen) remains active and functional in CRPC disease. We are studying the mechanism through which AR becomes functional in prostate cancer patients exhibiting CRPC disease. Emergence of CRPC phenotype depends on different mechanisms, such as activation of receptor tyrosine kinase, uncontrolled cell growth, and genomic mutation of AR that allows response to nonspecific AR-ligands. We are testing whether isoforms or splice variants of androgen receptors play a role in the CRPC disease. It has been reported that AR splice variants activate genes involved in the metabolism of androgens and provide a survival advantage for cells in a low-androgen environment. Our laboratory has identified the mechanism through which AR-variants induce their pro-growth activity in tumor cells. Notably, we have identified an agent that inhibits the activity of AR-variants in CRPC cells. The validation of this mechanism-based agent in animal models is expected to provide an excellent alternative or adjuvant modality for the treatment of advanced prostate cancer, particularly of the CRPC phenotype.

6. Investigating the causes of racial disparity in prostate cancer

According to the American Cancer Society, the higher overall cancer death rate among African American men is due largely to higher mortality rates from prostate, lung, and colorectal cancers. Although the overall racial disparity in cancer death rates has decreased, the death rate for all cancers combined continues to be 32% higher in African American men than in Caucasian men. African American men with prostate cancer have worse disease with a higher incidence, are younger in age with more advanced disease at diagnosis, and have a worse prognosis compared to Caucasian men. In addition to socioeconomic factors and lifestyle differences, molecular alterations have been reported to contribute to this discrepancy. Recent developments in genetics, proteomics, and genomics, among other molecular biotechnologies, are anticipated to greatly aid in the advancement of translational research on prostate cancer racial disparity and hopefully will culminate in the discovery of novel mechanisms of disease in addition to prognostic markers and novel therapeutic approaches. The research project running in our section is aimed at investigating the molecular mechanisms that cause the failure of cancer treatment for African American men. Though widely used in clinics, PSA has been reported to be insufficient as a reliable biomarker for prognosis of prostate cancer in African American men. The larger aim is to identify novel biomarkers that could be used for prostate cancer prognosis in Caucasians as well as in African American men. We recently showed that BMI-1, a stem-cell protein, could be developed as a sensitive and reliable blood-biomarker for prostate cancer disease in Caucasian and African-American men.

7. Lupeol, a dietary triterpene: testing its efficacy for the prevention and treatment of prostate, pancreatic and colon cancer

Another major goal of our laboratory is to identify novel and non-toxic agents that could be developed as chemopreventive and chemotherapeutics agents for either inhibiting cancer development or treating cancer in humans. We have identified a non-toxic compound called "lupeol" that exhibits the potential to be developed as a chemopreventive and chemotherapeutic agent against cancer. Lupeol, a fruit- and vegetable-based triterpene, is found in olives, grapes, cucumbers, berries, and mangoes as well as in herbs, such as aloe vera. Our laboratory has shown that lupeol application on skin prevents cancer development in animal models. We further have shown that lupeol treatment inhibits the growth of prostate, pancreatic, and skin tumors (of human origin) using relevant mouse models. These studies have generated interest in studying lupeol for other cancer types. We also recently observed that lupeol has the potential of improving chemotherapy in colon cancer. Our pharmacokinetic studies have shown that lupeol is bioavailable in relevant mouse models after consumption (as oral administration).

8. Testing cocoa polyphenol (dark chocolate)-based functional foods in the prevention and treatment of cancer

Functional food is any healthy food claiming to have a health-promoting or disease-preventing property beyond the basic function of supplying nutrients. Functional chocolate consumption has been associated with improvements in delayed oxidation of low-density lipoprotein cholesterol and lowered blood pressure in humans. Cocoa-based chocolate consumption has been associated with short-term improvements in delayed oxidation of low-density lipoprotein cholesterol, improved endothelial function, lowered blood pressure, and better platelet function. Epicatechin is the major component of cocoa powder. We have employed a technique (called ACTICOA) that provides the cocoa polyphenol powder highly rich in epicatechin content. Our studies show that epicatechin-rich cocoa polyphenol selectively inhibits growth of premalignant prostate and pancreatic cells while sparing normal cells via modulation of NFκB signaling pathway. We are testing cocoa polyphenol in animal models evaluating its preventive as well as therapeutic value against cancer. For our studies, we have collaborated with Barry Calibaut (Belgium), one of the leading companies in the world that produce functional foods, including functional chocolates.

Research partner institutions

Our section has joined hands with internationally renowned research institutions and investigators in our quest to defeat the lethal disease of cancer in humans. Studies are underway in partnership with the following research institutions:

1. Institute for Cancer Biology, Danish Cancer Society, Copenhagen, Denmark
2. Research Center for Advanced Science and Technology, University of Tokyo, Japan
3. Mayo Clinic, Rochester, MN, USA
4. Roswell Park Cancer Institute, Buffalo, NY, USA
5. Center for Prostate Disease Research, Uniformed Services University of the Health Sciences, Bethesda, MD, USA
6. Albert Einstein College of Medicine, Bronx, NY, USA
7. University of Illinois-Chicago, IL, USA
8. Clark-Atlanta University, Atlanta, GA, USA

Other Professional Activities

Mohammad Saleem

Scientific expert in review panels of grant funding agencies (national & international)

Molecular Biology panel on Prostate cancer awards (CDMRP) Department of Defense

Pathology Biomarkers panel on Prostate cancer awards (CDMRP) Department of Defense

Rolex Research Awards, Rolex Corporation, Geneva, Switzerland

Arthritis-Research UK, United Kingdom

Prevention panel on breast cancer awards (CDMRP) Department of Defense

Special Emphasis Panel (ZCA1 SRLB-J (O1)S) National Cancer Institute, NIH

Adhoc-reviewer of Scientific Journals

Journal of Biological Chemistry, Oncogene, Neoplasia, Cancer Research, Clinical Cancer Research, Oncotarget, PLOSE-one, Biochemical Pharmacology, Biochemica Biophysica Acta, Melanoma Pigment Research, Cancer Letters, Toxicology Applied Pharmacology, Life Sciences, Photochemistry and Photo Biology, Chemosphere, Clinica Chemica Acta, Molecular Cellular Biochemistry, Phytotherapy Research, Journal of Pharmacy and Pharmacology, Food Chemical Toxicology, Molecular Carcinogenesis, International Journal of Cancer, Molecular Cancer Therapeutics, Carcinogenesis, British J of Breast Cancer

Editorial Board Member

PLOSE ONE

American Journal of Stem Cell

Nutrition and Medicine

American Journal of Clinical Experimental Urology

CANCER BIOMARKERS AND DRUG RESISTANCE

Associate Director / Section Leader
Ann M. Bode, Ph.D.
Professor

Immunohistological staining of Cox2 in squamous cell carcinoma

"Our work funded by NIH has focused on biomarker identification in breast and bladder cancer."

Dr. Ann M. Bode

Our laboratory is funded by a National Institutes of Health (NIH) contract and Pediatric Pharmaceuticals. The purpose of the NIH studies is to identify and measure specific cellular signal transduction endpoints with the purpose of identifying potential biomarkers and mechanisms of action of the various anticancer agents. The work funded by Pediatric Pharmaceuticals focuses on the anti-skin cancer effects of ginger compounds.

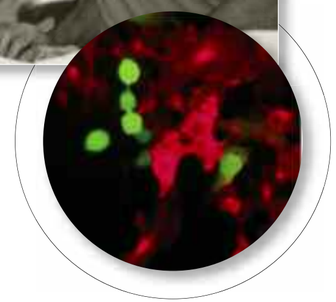
The NIH funded work focuses on studies with mammary tumors and normal mammary glands, and effects of chemopreventive agents in in vivo mammary models. The primary purpose of this work is to determine whether specific signal transduction molecules can serve as reliable endpoint biomarkers for clinical trials as well as provide insight into the mechanism(s) by which selected anticancer agents modulate their preventive effects. The development and identification of reliable biomarkers will allow us to 1) assess the efficacy of potential chemopreventive or therapeutic agents for clinical trials; 2) identify patients who will respond to specific drug treatments; and 3) determine the mechanisms of action of specific agents or mixtures of agents (e.g., food mixtures). These are major objectives in the field of chemoprevention and cancer therapeutics. To identify biomarkers, we use a variety of techniques, including immunohistochemisty, Western blot analysis, protein array analysis, and cell culture. The use of Neu expressing or ER positive breast cancer cell lines to test the effect of RXR inhibitors, targretin and UAB, on migration and invasion, along with other characteristics of cancer, is being investigated.

Our work funded by Pediatric Pharmaceuticals has focused on developing a ginger-based formulation to treat and/or prevent solar UV-induced skin cancer.

Left to right: Ann M. Bode, Alyssa Langfald
Not pictured: Hongxun Wang

MEMBRANE BIOCHEMISTRY

Section Leader
Rhoderick E. Brown, Ph.D.
Professor



Cholera toxin B endosytosis was affected by the overexpression of GLTP.

Cells envelope their contents by means of thin, flexible barriers called "membranes" that enable selective import of nutrients and export of toxic by-products. Assembly of membranes relies on specialized lipids that are polar at one end and nonpolar at the opposite end. With the polar ends preferring to be in contact with water and the nonpolar ends wanting to avoid water, these lipids readily orient and arrange themselves as thin, flexible layers only two-molecules thick, i.e. bilayers. These membrane bilayers not only surround cells but also form internal partitions that enable regions of specialized function inside of cells. Interestingly, there are many more varieties of lipids found in membranes than are needed to form bilayers. Some membrane lipids also can function as messenger signals that regulate cell growth, proliferation, and programmed-cell death processes, while other membrane lipids appear to cluster together in bilayers to form microdomains that are able to control the spatial distribution and lateral interactions of certain membrane proteins. The discovery of these new functions for membrane lipids underscores the reasons why biomembranes come under direct attack during cancer and infectious disease.

Our research is focused on a membrane lipid class known as "sphingolipids and proteins" that can bind and transfer sphingolipids between membranes. Certain sphingo-lipids, along with cholesterol, serve as key components needed for formation of membrane "raft" microdomains. Rafts appear to function as organizing regions for certain signaling kinases as well as target sites for certain viruses and bacteria. Earlier efforts focused on rigorously defining the physical basis for raft microdomain functionality. To do so, we developed ways to quantitatively measure the lateral elasticity within model membranes to accurately assess the physical changes that occur within the membrane "raft environment" as the content and structure of sphingolipids and sterols are systematically altered. Our research revealed structural features of sphingolipids that regulate their interactions with other membrane lipids and provided insights into the unique physical features in membrane microdomains at the heart of their lateral organizing functionality for spatially organizing and regulating various proteins that interact with membranes.

Formation and maintenance of sphingolipid-enriched microdomains are likely to involve specific proteins that can bind and transfer sphingolipids between membranes. Much effort in our lab has been directed toward a protein family known as "glycolipid transfer proteins" (GLTPs) that can specifically bind and transfer glycosphingolipids between membranes. We found that GLTP functionality is regulated by lipid composition and packing within membranes. To gain insights into the lipid structural features that control both the lateral and transmembrane distributions of sphingolipids, we use a combination of biophysical approaches (fluorescence spectroscopy, Langmuir surface balance approaches, calorimetry, NMR). We apply this basic knowledge to help decipher how changes in membrane lipid composition regulate GLTP function. Our molecular biological studies resulted in the first molecular cloning of human GLTP and showed that related homologs originating from mRNA transcripts exist in mammals, plants, and fungi. Genetic-engineering approaches enable production of human GLTP and related homologs in bacterial expression systems and purification of sufficient quantities to crystallize the proteins as well as determine their conformational structures both in glycolipid-free form and complexed with different glycolipid, in collaborative studies with structural biologists at Memorial Sloan Kettering Cancer Center in New York. We discovered that human GLTP forms a novel structural fold among known proteins. As a result, the Protein Data Bank in Cambridge, England, designated human GLTP as the founding member and prototype of the new GLTP superfamily, enabling our research findings to be published in Nature, PLoS Biology, Structure, The Journal of Biological Chemistry, Biophysical Journal, and Biochemistry. Our studies have revealed: i) how GLTP adapts to accommodate different glycolipids within its binding site; ii) the functional role played by intrinsic tryptophan residues in membrane interaction and glycolipid binding; and iii) the structural basis for the narrower glycolipid selectivity of a fungal GLTP ortholog.

In recent investigations also published in Nature, we have reported the discovery of a new GLTP structural homolog in human cells that we named "ceramide-1-phosphate transfer protein" (CPTP). Remarkably, the lipid specificity of CPTP has evolved for transferring ceramide-1-phosphate rather than glycolipids even though CPTP still forms a GLTP-fold, albeit encoded by a different gene than GLTP. With collaborating investigators at Virginia Common¬wealth University, we have shown that, when human cells become depleted of CPTP, newly synthesized ceramide-1-phosphate accumulates in the Golgi. The over-accumulation triggers the action of cytoplasmic phospholipase A2 responsible for generating arachidonic acid that becomes further metabolized into pro-inflammatory eicosanoids.

> "The discovery of these new functions for membrane lipids underscores the reasons why biomembranes come under direct attack during cancer and infectious disease."
>
> *Dr. Rhoderick E. Brown*

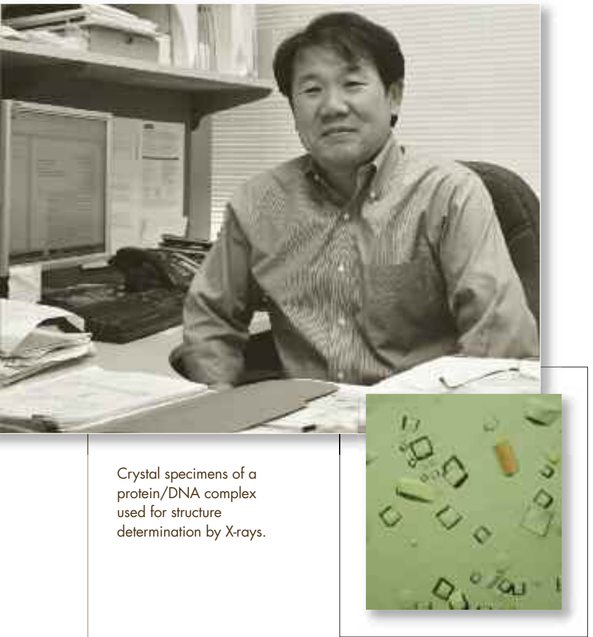
We anticipate that elucidation of the fundamental structure-function relationships governing the action of human GTLP, CPTP and related homologs will facilitate development of the means to pharmacologically modulate these proteins as well as enhance their potential development as biotechnological resources, i.e. nanotools, for manipulation of targeted sphingolipid levels in cells. Such strategies could provide new ways to introduce specific sphingolipid antigens to help achieve the targeted destruction of cancer cells via immunotherapeutic means, and lead to new therapeutic approaches to treat disease processes involving sphingolipids.

Our exciting progress to date emphasizes the need for continuing studies into the workings of GLTP, CPTP, and other proteins containing GLTP-like motifs using comprehensive strategies involving biophysical, cell, and molecular biological approaches. Our recent investigations of the gene organization and transcriptional status in humans as well as other mammals now provide a firm foundation for the identification and characterization of inherited diseases involving GLTP and CPTP. Our ongoing efforts benefit from collaborations with researchers at Memorial Sloan Kettering Cancer Center in New York; Virginia Commonwealth University in Richmond; The Russian Academy of Sciences in Moscow; CIC bioGUNE in Derio; Spain; and the Mayo Clinic. Our research continues because of financial support received from the National Institute of General Medical Sciences; the National Cancer Institute of NIH; and The Hormel Foundation.

Left to right: Rick Brown, Xiuhong Zhai, Liudmila (Lucy) Malinina, Helen Pike, Shrawan Kimar Mishra

STRUCTURAL BIOLOGY

Section Leader
Young-In Chi, Ph.D.
Assistant Professor



Crystal specimens of a protein/DNA complex used for structure determination by X-rays.

Structural biology is a branch of biomedical science concerned with molecular structures of biological macromolecules, such as proteins and nucleic acids. Due to their biological functions being tightly coupled to their molecular structures, elucidating atomic details of their structures – either alone or in complex with functional binding partners – is crucial to understanding the molecular mechanisms underlying their physiological functions. Biomolecules are too small to see even with the most-advanced electron microscope. Special techniques, therefore, need to be employed, and we particularly harness X-ray crystallography as a main experimental tool to elucidate three-dimensional structures. This technique involves various disciplines of modern biomedical research, such as molecular biology, protein chemistry, biophysics, and various computations. We also perform diverse functional studies to complement the structural studies.

Our research currently is focused on elucidating the atomic details of key molecular interactions involved in human diseases, especially diabetes and cancer. We, in particular, are focusing on (i) transcriptional regulators involved in diabetes and (ii) protein functional modulators involved in tumor progression and metastasis. We apply structural biology to gain a better understanding of their normal function and dysfunction in the disease state as well as an opportunity to discover or design structure-based functional modulators.

HNF1α (Hepatocyte Nuclear Factor1α) and HNF4α are the master regulators of pancreatic β-cell development and function, and their mutations are the most-common monogenic causes of diabetes referred to as MODY. Over the years, we have determined the crystal structures of the functional complexes made by HNF1α and HNF4α. These structures provided valuable information on the molecular basis of target gene recognition, ligand-mediated activation, and functional disruption by disease-causing mutations. These structures, however, provided only partial answers as to how their full transcriptional activities arise, and how these proteins are involved in additional protein-protein interactions and physiological functions. Therefore, we set out to identify previously unknown functional binding partners of HNF1α and HNF4α in β-cells, and study the physiological implications of these interactions, especially on insulin secretion that is impaired in MODY patients. We performed structural studies of the complexes and functional characterization of MODY mutations. We previously published the findings on the mediator component of the main transcriptional machinery, MED25, as a functional binding partner of HNF4α and its implication to β-cell function. We currently are in the process of following-up additional binding partners and their physiological implications, such as novel transcriptional co-repressors AES and EBP1 for HNF1α and HNF4α, respectively. The findings from these studies will advance the current understanding of the transcription regulatory network in β-cells and provide a new avenue for diabetes treatment/prevention strategies by discovering novel and more effective target sites for designing and further improving partial agonists selectively against them.

One other diabetes-related project we have been working on is the structural basis of Glucose-6-phosphatase (G6pase) gene regulation, especially by the transcription factors Foxo1 and Creb. G6pase is a key regulating enzyme for gluconeogenesis in the liver and considered to be an attractive target for diabetes treatment. Last year, we finally finished the Foxo1/DNA complex structure and now have submitted the manuscript for publication, in which we have identified a new Foxo1 binding site and novel biding modes on G6pase promoter.

As part of cancer-related projects, Dub3 is an ubiquitin hydrolase (de-ubiquitinase) and a key protein that relays extrinsic signals to regulate epithelial-mesenchymal transition (EMT) and metastasis in breast cancer, which can serve as a druggable target for treating triple negative/basal-like breast cancers. To gain the ground work for structure-based rational drug design against this protein, we set out to determine the crystal structure determination of the Dub3 catalytic domain alone and/or its complex ubiquitin, its substrate. We made sufficient progress last year and are in the process of improving the crystals and finishing up the structure determination. Upon completion of the structures, we will conduct a computer-aided docking analysis of chemical library compounds to discover/design specific inhibitors of Dub3 to improve the prognosis of these hard-to-treat breast cancers. Candidate compounds will be purchased and tested in vitro and in vivo for their ability to suppress the de-ubiquitinase activity of Dub3. These findings will validate the effectiveness of Dub3 target strategy and could open new doors for therapeutic intervention.

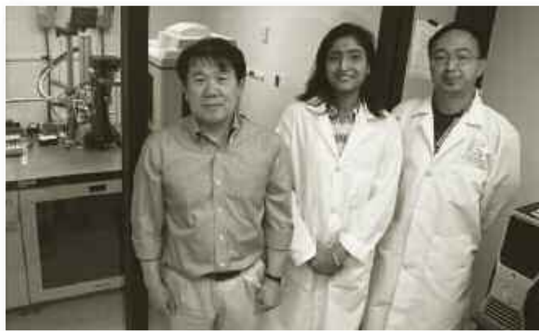
> "Our research currently is focused on elucidating the atomic details of key molecular interactions involved in human diseases, especially diabetes and cancer."
>
> *Dr. Young-In Chi*

Additional cancer-related projects in the lab with therapeutic values include FABP/inhibitor complexes, leukemia-associated histone modifiers, novel protein kinases/inhibitor complexes, and small RNA molecules for drug delivery. Research in our laboratory will continue to this end, and target molecules will be expanded to include more cancer-related proteins that will lead to additional preliminary data for sustaining grant applications. Crystal structure determination, functional studies, and drug discovery will provide a critical basis for human physiology, dysfunction in the disease state, and a better strategy for therapeutic intervention.

Other Professional Activities
Young-In Chi

Invited speaker:
University of Minnesota BICB program Seminar Series

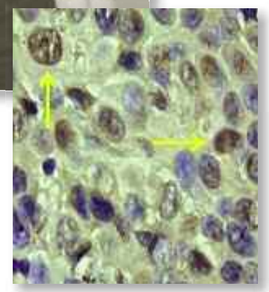
Ad-hoc Journal Article Review:
Biochemistry
Journal of Endocrinology & Diabetes
Journal of Diabetes Research
Acta Crystallographica section F



Left to right: Young-In Chi, Puja Singh, Shu-Ping Tung
Not pictured: Eun Hee Han

NUTRITION AND METABOLISM

Section Leader
Margot P. Cleary, Ph.D.
Professor



Pictured is an estrogen receptor from a series of images showing the staining of mammary tumors for adipokine (made in fat tissue) growth factors. Brown staining are the proteins of interest.

Primary interests of the Nutrition and Metabolism section are the effects of body weight and food intake on the development of breast cancer using mouse models. Past studies have included effects of genetic and dietary induced obesity on breast/mammary tumor development, particularly with respect to body fat and serum IGF-I, leptin and adiponectin levels. Other studies have assessed the effect of calorie restriction on the prevention of mammary tumors in several mice models of breast cancer. Of particular interst, we consistently find that periods of moderately severe calorie restriction followed by refeeding – which we term "intermittent calorie restriction" – results in much greater reduction in mammary tumor incidence than the same degree of restriction implemented chronically with both interventions resulting in 20-25% calorie reduction. Mechanisms of the protective effect of caloric restriction on cancer development include studies of leptin/leptin receptors, adiponectin/adiponectin receptors and the IGF-axis. Based on results of our studies, we have hypothesized that the altered i.e., reduced adiponectin:leptin ratio, which is characteristic of obesity, provides a permissive environment for tumor development. In contrast, the reductions of IGF-I and leptin and increased adiponectin:leptin ratio resulting from intermittent calorie restriction results in reduced mammary tumor development and decreased mammary tumor incidence in comparison to ad libitum feeding as well as chronic calorie restriction. These studies have been expanded by Dr. Michael Grossmann to include the interaction of an omega-3 fatty acid in combination with intermittent calorie restriction on the development of mammary tumors. The intermittent calorie restriction approach may provide an easier approach for individuals to reduce caloric intake for disease prevention.

Although calorie restriction has an incredible effect on cancer prevention in rodent models of many kinds of cancer, the practical aspects of implementing and maintaining this intervention in human populations has not been very successful. This has led to interest in identifying compounds that act like calorie restriction (i.e. calorie restriction mimetics). One such compound is the commonly used type 2 diabetic drug, metformin. Our most recent work focuses on directly comparing moderate calorie restriction (25% reduction) to metformin treatment on the prevention of mammary tumors. This study is being conducted in a transgenic mouse model to mimic postmenopausal breast cancer and includes obese as well as normal-weight subjects. The intervention is being started when the mice are middle-aged to also reflect what would occur in at-risk women. We also are conducting studies related to the effects of metformin on cancer progression. With respect to mechanisms of action of these interventions, not only are we assessing alterations in the AMPK pathway but also on aspects of altered glucose metabolism that may result. We anticipate that these ongoing studies will provide valuable insights into ways to prevent mammary tumor development and to slow disease progression.

Other Professional Activities
Margot P. Cleary

Invited speaker
Georgia Regents University- Cancer Center

Presentations
4th Annual Masonic Cancer Center Research Symposium (Dr. Yang)

Attended
AACR 12th Annual Frontiers in Cancer Prevention Meeting – Washington, DC

Grant Review Committees
3 NIH Study Section Meetings (July, October and May)

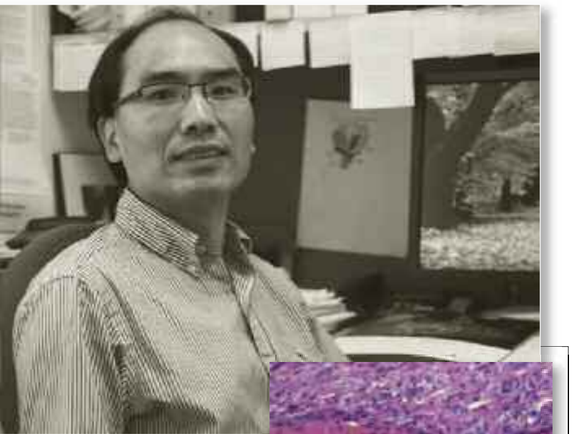
"We anticipate that these ongoing studies will provide valuable insights into ways to prevent mammary tumor development and to slow disease progression."

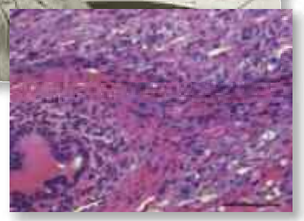
Dr. Margot P. Cleary

Left to right: DaQing Yang, Shuxia (Susan) Jiang, Michael Grossmann, Margot Cleary, Ben Harris, Nancy Mizuno, Defeng Wang.

CELL DEATH AND CANCER GENETICS

Section Leader
Yibin Deng, M.D., Ph.D.
Assistant Professor





Loss of tumor suppressor genes Pten and p53 in prostate epithelial cells cause castration-independent prostate cancer in genetically engineered mouse models.

The TP53 gene encodes a tumor suppressor protein that functions as a stress response and cell cycle checkpoint protein to maintain genomic integrity. The importance of p53 in tumor suppression is highlighted by mutations that lead to the loss of wild-type p53 activity and/or gain of oncogenic function found in more than half of human cancers. The recent comprehensive genomic sequencing studies sponsored by The Cancer Genome Atlas (TCGA) consortium confirm the high frequency of TP53 mutations in many sequenced human cancers. TCGA studies, for example, found that 96 percent of ovarian cancers, 37 percent of breast cancers, 54 percent of colorectal cancers, and 81 percent of lung squamous cell carcinomas displayed TP53 mutations. How wild-type p53 inhibits tumor development and why mutant p53 promotes tumor progression and/or metastasis, however, remains incompletely understood.

Our laboratory, therefore, focuses on understanding the molecular mechanisms by which wild-type p53 suppresses tumorigenesis and the gain-of-function properties of mutant p53 that promote tumor progression and metastasis. To translate our bench work to bedside, we utilize genomic and proteomic approaches, bioinformatics, computational modeling, RNAi-based screening, and genetically engineered mouse models (GEMMs) that recapitulate the salient features of human cancers to discover the crucial "druggable" targets for cancer cells. Our ultimate goal is to identify molecular targets, small molecular compounds and therapeutic drug combinations that selectively and efficiently kill cancer cells harboring "unique" genetic alterations identified in patients while leaving the normal cells unharmed. In the past year, our laboratory has made progress in the following three major areas:

1. Understanding p53-initiated signaling pathways in tumor suppression in vivo

While many studies have focused on the role of apoptosis and/or senescence in p53-mediated tumor suppression, recent findings suggest that p53 induces DRAM (Damage-Regulated Autophagy Modulator)-dependent autophagy. The function of p53-DRAM-mediated autophagy in tumorigenesis, however, remains unknown. To study the role of DRMA-mediated autophagy in tumor development, we generated conditional DRAM knockout mice. Our findings suggest that DRAM potentially functions as a tumor suppressor because deletion of DRAM promotes spontaneous tumor development in mouse models. We currently are dissecting the molecular basis underlying DRAM-deficiency driven tumorigenesis in vivo. We also are exploring how the crosstalk between p53-initiated autophagy and p53-mediated cell metabolism leads to tumor initiation, progression and metastasis.

To answer the critical function of p53-mediated autophagy, apoptosis and senescence in suppressing tumor development in vivo, we have generated "triple" mutant mice utilizing the conditional DRAM knockout mice to breed with mice deficient in p53-mediated apoptosis (p53R172Pknock-in or PUMA knockout) and senescence-deficient mice (p21 knockout). We expect that by utilizing these complex genetically engineered mouse models, we will be able to identify crucial molecular targets contributing to p53-mediated tumor suppressive function in vivo.

2. Gain-of-function of mutant p53 in telomere uncapping-driven breast tumorigenesis

Human sporadic breast carcinomas are characterized by the presence of complex cytogenetic aberrations. One of the foremost challenges for breast cancer researchers is to develop experimental model systems that identify pathogenetic events driving breast tumor development. Our long-term goal in this project is to establish "chromosomal instability" mouse breast cancer models and discover the "causal" genomic events driving breast tumorigenesis in vivo. One important mechanism that can give rise to the unstable breast cancer genome is the dysfunction of telomeres. Telomeres are nucleoprotein caps that protect chromosomal ends from being recognized as damaged DNA and prevent chromosome end-to-end fusions. Telomeres that no longer can exert end-protective functions are said to be dysfunctional, and these 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). In human breast carcinomas, chromosomal instability fueled by dysfunctional telomeres is associated with the transition from benign ductal hyperplasia to malignant ductal carcinoma in situ. This strongly supports the notion that telomere dysfunction-induced chromosome instability initiates the development of breast cancers. Our laboratory has been engineering a novel mouse breast cancer model harboring telomere uncapping-induced chromosomal instability without affecting the activity of telomerase. In addition, the mouse model also expresses "hot spot" mutant p53 protein in breast epithelium. We believe that this mouse model will faithfully recapitulate the genetic abnormality commonly observed in human sporadic breast carcinomas. We are establishing and utilizing this novel mouse breast cancer model to identify the key genetic pathways perturbed in chromosomal instability driven mammary tumorigenesis and target these pathways with novel therapeutics that potentially could suppress human breast cancer.

Our studies currently suggest that endogenous expression of mutant p53 promotes breast tumor development in comparison with the loss of p53 in breast epithelial cells. We also are using genetic and pharmacological approaches in genetically engineered mouse models to verify our cell-based studies in vitro and uncover the crucial targets contributing to mutant p53 gain-of-function in promoting tumor progression and metastasis in vivo.

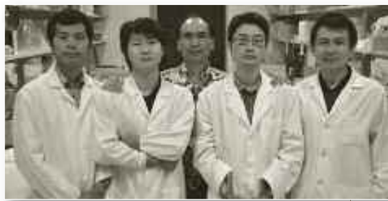
3. Exploring the molecular targets involved in selective killing of cancer cells

Our laboratory has a long-standing interest in understanding genetic pathways that allow for selective targeting of cancer cells while leaving normal cells unharmed. Through multidisciplinary collaborations, we are using a comprehensive combination of genomic and proteomic analyses of cancer, computational modeling of cancer pathways, RNAi-based screening, and functional testing in cell culture and animal models of cancer to identify the crucial signaling axis that are linked to therapeutic selectivity.

We recently have made progress in our study on prostate cancers. Prostate cancer is the second-leading cause of cancer-related death in males after lung cancer and strikes one in six men in the United States. Recent advances in whole genome and exome sequencing suggest that co-deletions of tumor suppressors Pten and p53 occurs frequently in lethal human castration-resistant prostate cancer (CRPC). Genetic studies in mouse models support that Pten and p53 co-deletions play a causal role in the development of CRPC in vivo. Thus, finding effective and selective means of killing prostate cancer cells carrying Pten/p53 deficiency is critical to successfully treating currently incurable CRPC. This strategy, however, is limited by the scarcity of identified crucial targets required for Pten/p53 deficiency driven CRPC. We used bioinformatics and genetic approaches to find that hexokinase II (HK2) – which catalyzes the first committed step in glucose metabolism – is exclusively expressed in human prostate cancer cells, particularly elevated in human prostate cancer harboring Pten/p53 deletions. Mechanistically, deletion of Pten increases HK2 mRNA translation through the activation of AKT-mTOR-4EBP1 axis and loss of p53 enhances HK2 mRNA stability through inhibition of miR-143 biogenesis. Genetic and pharmacological studies demonstrated that HK2-mediated aerobic glycolysis, known as the Warburg effect, is required for tumor growth of human CRPC cells harboring Pten/p53 deficiency in xenografts model. Whereas targeting HK2-initiated glycolysis activates AMPK-dependent autophagy for cell survival, and pharmacologically co-targeting glycolysis with 2-deoxyglucoe (2-DG) and autophagy with chloroquine (CQ) selectively induces cancer cell apoptosis to cause near-complete tumor remission in the Pten/p53 deficiency driven CRPC mouse models. Our findings, thus, provide a potential therapeutic strategy for the patients harboring mutant Pten and p53. Part of the work has been published in Cell Reports. Our laboratory also is utilizing multiple genetic and pharmacological approaches to identify targets that can be selectively targeted in human lung and colon cancers.

"Finding effective and selective means of killing prostate cancer cells carrying Pten/p53 deficiency is critical to successfully treat currently incurable CRPC."

Dr. Yibin Deng



Left to right: Huanan Wang, Yingjie Zhang, Yibin Deng, Ji Wang, Lei Wang. Not pictured: Fengxia Wu, Li Chang, Tao Lin, Zhiqi Yu.

Our ongoing collaborations are with researchers from Texas Tech University Health Sciences Center School of Pharmacy in Amarillo, TX; The University of Texas M.D. Anderson Cancer Center in Houston, TX; Roswell Park Cancer Institute in Buffalo, N.Y.; and Mayo Clinic College of Medicine in Rochester, MN. Our research projects are supported by the grants from National Cancer Institute of NIH and The Hormel Foundation.

Other Professional Activities
Yibin Deng

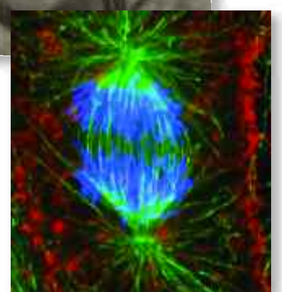
Invited speaker, Mayo Clinic College of Medicine
Grant Reviewer, National Cancer Institute

CELLULAR DYNAMICS

Section Leader

Edward H. Hinchcliffe, Ph.D.

Associate Professor



Ovarian cell with multiple centrosomes, mimicking a pre-cancerous state – microtubules in red, centrosomes in green, chromosomes in blue.

Our section studies the molecular mechanisms that regulate normal cell division, the roles played by duplication of the centrosome in ensuring the bipolarity of this process, and defects in chromosome segregation (the gain/loss of whole chromosomes during mitotic division, which is associated with tumor progression).

Cell division lies at the heart of normal tissue development and maintenance. The division of cells must occur in a strict one-to-two fashion to ensure genomic stability. The loss or gain of whole chromosomes during abnormal cell division leads to aneuploidy in which daughter cells have variable chromosome numbers. This is a major problem for cells because there is a change in the dosage of essential gene products. The cell has developed multiple, biochemical checkpoints and fail-safe devices to ensure that cell division occurs with absolute fidelity. Unfortunately, DNA mutations – often caused by environmental factors – can render these molecular, quality-control mechanisms inoperable. The result is the inadvertent missegregation of chromosomes during cell division, leading to genomic abnormalities and tumorigenesis.

Chromosome instability (CIN) is a hallmark of solid tumors and contributes to the genomic heterogeneity of tumor cells. There are multiple mechanisms believed to underlie the generation of CIN, including cell-cycle defects; abnormal centrosome duplication and function; premature chromatid disjunction; and centrosome-separation errors. Despite an increasingly mechanistic understanding of how CIN is generated, however, we know relatively little about how chromosome missegregation becomes transduced into cell transformation and tumorigenesis. A major unresolved question is the role of cell-cycle checkpoints and fail-safe devices in preventing chromosome missegregation in the first place. At the heart of this issue is the question of how a single, missegregated chromosome can trigger the p53/p21 pathway and induce durable cell-cycle arrest – a molecular fail-safe device that monitors aneuploidy and prevents the proliferation of aneuploid cells. Current work focused on DNA damage caused by lagging chromosomes is part of the answer. To date, however, no mechanisms have been identified that monitor chromosome mispositioning – either before or after anaphase – at the single chromosome level.

The centrosome is an organelle that nucleates and organizes the microtubule cytoskeleton. This, in turn, is used to build the bipolar mitotic spindle, which is responsible for aligning and segregating the duplicated chromosomes during cell division. Centrosomes are thought to play a major role in establishing the bipolarity of the mitotic spindle. To ensure this, the single centrosome normally duplicates exactly once during the cell cycle, yielding a pair of centrosomes that form the two spindle poles. In many cancer cells, the number of centrosomes increases, resulting in a small but significant number of cells with more than two spindle poles and an increase in the probability of abnormal cell division. It is, therefore, important to understand the molecular mechanisms that drive normal centrosome duplication, and restrict centrosome duplication to once per cell cycle.

In our lab, we use cultured mammalian cells and cytoplasmic extracts generated from Xenopus frogs to examine the basic control mechanisms underlying centrosome duplication, cell division, and cytokinesis. We use advanced imaging techniques, such as live-cell confocal fluorescence microscopy, Fluorescence Recovery After Photobleaching (FRAP), microinjection and microsurgery, to address these fundamental questions in cell biology. Our research has direct relevance to understanding the underlying mechanisms that lead to cancer initiation and progression. Our work also is relevant to identifying potential targets for chemotherapy agents.

> "Mistakes in the cell-division process can have disastrous consequences for the cell, leading to aneuploidy, cellular transformation and tumorogenesis."
>
> *Dr. Edward H. Hinchcliffe*

Experimental research results

1. Coordinating cytokinetic furrow formation with anaphase onset

The cell division furrow – created by the recruitment of actin filaments and the motor protein myosin II – is formed between the separating sister chromatids at anaphase. This furrow constricts the dividing cell into two daughters. To ensure that cytokinesis occurs in the right place and at the right time, the positioning of the cleavage furrow must be coupled to the segregation of the chromosomes. This occurs through signaling via the microtubule network, specifically the dynamic astral microtubules and the stable, overlapping midzone microtubules. Both of these classes of microtubules are important for signaling the formation of the cytokinetic furrow and ensuring that the furrow remains restricted to the cell center. We are investigating the regulation of furrow formation using live-cell imaging and single-cell manipulation. We are taking advantage of the fact that microtubules are extremely sensitive to temperature and can be disassembled by cold treatment without causing harm to the cell. When the cells are warmed up, the microtubules re-assemble and the cell cycle proceeds on its way. Using this system and spinning disk confocal microscopy, we are able to examine the roles of candidate regulatory mechanisms, including Aurora B kinase, Polo-like kinase 1, and the relative contributions of the astral and midzone microtubules. Our goal is to integrate molecular studies with live-cell physiology to understand the mechanisms underlying cell division.

We have found that there is a period following the onset of anaphase in which the cell cortex can respond to furrow-inducing signals, and that this period is sensitive to the loss of microtubules and the activity of Polo-like kinase 1. Once cells progress beyond this point, however, the furrow will form, regardless of whether microtubules persist. Polo-like kinase 1 activity also is not required after this "point of no return." Adding kinase inhibitors after this point does not affect the ability of a furrow to assemble.

Left to right: Edward Hinchcliffe, Kul Karanjeet

2. Centrosome duplication

When CHO cells are arrested in S-phase, they undergo repeated rounds of centrosome duplication without cell-cycle progression. While the increase is slow and asynchronous, the number of centrosomes in these cells does rise with time. To investigate mechanisms controlling this duplication, we have arrested CHO cells in S-phase for up to 72 hours and inhibited centriole formation by treatment with the microtubule poison colcemid. We find that, in such cells, the pre-existing centrosomes remain and a variable number of centrosomal foci – containing α/γ-tubulin and centrin 2 – assemble at the nuclear periphery. If the colcemid is washed out, these nuclear-associated centrosomal foci disappear.

In their place, multiple centriole-containing centrosomes assemble, which nucleate microtubule asters and can form functional mitotic spindle poles. The eventual number of centrosomes that assemble following colcemid washout increases with time, even though that does not occur for the number of nuclear-associated centrosomal foci. Importantly, the number of centrosomes that assemble following colcemid washout corresponds to the number of centrosomes that form during S-phase arrest alone. This suggests that during S-phase, a core centrosome activity repeatedly replicates, even if centrioles themselves are prevented from duplicating, and this activity has the capacity to dictate the assembly and copy number of new centrosomes.

3. Building a bipolar spindle

Mitosis must be carried out with high fidelity to ensure that each daughter cell receives a complete compliment of the genome. Mistakes in the cell-division process can have disastrous consequences for the cell, leading to aneuploidy, cellular transformation and tumorogenesis. The centrosome is known to play a critical structural role in the cell-division process. It organizes the microtubule network during interphase as well as astral microtubules at the spindle poles during mitosis.

We currently are using microsurgery coupled with time-lapse videomicroscopy of living acentrosomal cells to investigate the role of the centrosome in cell-cycle regulation. To directly visualize the role of microtubules and regulatory molecules during the acentrosomal cell cycle, we have generated a primate kidney cell line (BSC-1 cells) that constitutively express α-tubulin coupled to GFP. We find that, after several hours, acentrosomal cells reform their microtubule network into an organized array. Interestingly, the acentrosomal microtubule focus can separate into two, distinct poles prior to nuclear envelope breakdown. This demonstrates that the splitting of the microtubule network does not require a centrosome, contrary to previously held notions. We find, however, that in the absence of a centrosome, the splitting of the microtubule network is inefficient; approximately 40 percent of acentrosomal cells enter mitosis with a monopolar spindle. These cells cannot bipolarize, and they fail cytokinesis. Thus, there is some aspect of the centrosome that ensures that the microtubule network will split and separate before the onset of mitosis. It could be that acentrosomal microtubule focus is deficient in the recruitment of some key factor(s) necessary to ensure accurate splitting. This factor could be a regulatory activity, a structural activity or a combination of the two. It also is possible that the acentrosomal microtubule focus lacks sufficient microtubules to interact with the cell cortex. Regardless of the mechanism, our work reveals that centrosomes are absolutely necessary to ensure fidelity during mitotic-spindle assembly.

4. Tektin proteins: key to spindle poles and spindle midbodies

We currently are investigating the role of the tektin proteins in establishing the spindle midzone. Tektins first were identified as components of axonemal microtubules, where they are thought to impart structural rigidity and complex periodic spacing to these highly stable microtubules. Our recent results suggest that tektins localize to the overlapping microtubules at the spindle midzone, where they also play an important role. This region of the mitotic spindle is responsible for initiating cytokinesis and required for the process to continue. Many key regulators of late-mitotic events, along with cytokinesis, localize to the spindle midzone. When tektin 2 (one of three distinct tektins found in vertebrates) is knocked down using shRNAs, the midzone microtubules fail to become compacted and appear to exhibit abnormal, plus-end microtubule motility. The result is failure of cytokinesis.

In addition to the tektins, we are exploring the role of two, highly conserved proteins called EFHC 1 & 2. These Ca2+-binding proteins also are involved in centriole assembly and the formation of midbodies. Importantly, mutations in these proteins lead to abnormal cell division associated with neurological birth defects.

We are interested in uncovering the molecular mechanisms underlying these observations. We currently are examining the motility of several key regulators of midzone function – PRC1 and Kif4 – in response to experimental loss of tektin 2 and/or EFHC 1&2. We are using live-cell imaging and FRAP analysis to examine the role played by tektins in regulating these important components of the cell-division apparatus.

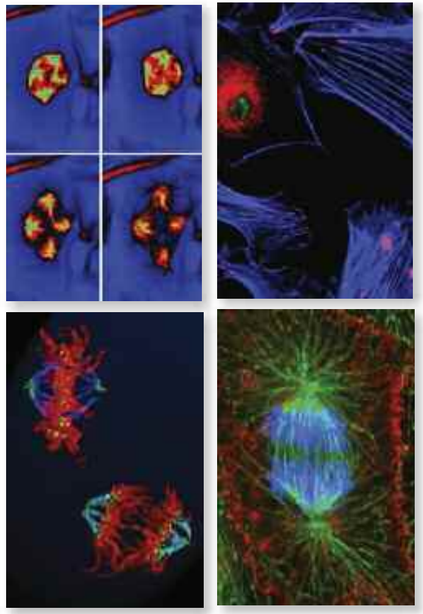
A detailed understanding of the regulation of cell division, cytokinesis and chromosome instability will advance our knowledge of the biology of cancer – itself a disease characterized by unregulated cell proliferation and chromosome missegregation. Our work will provide a mechanistic understanding of key cell-cycle events that may contribute to cancer progression. Together, these studies also will provide a source of potential targets for future anti-cancer drugs.

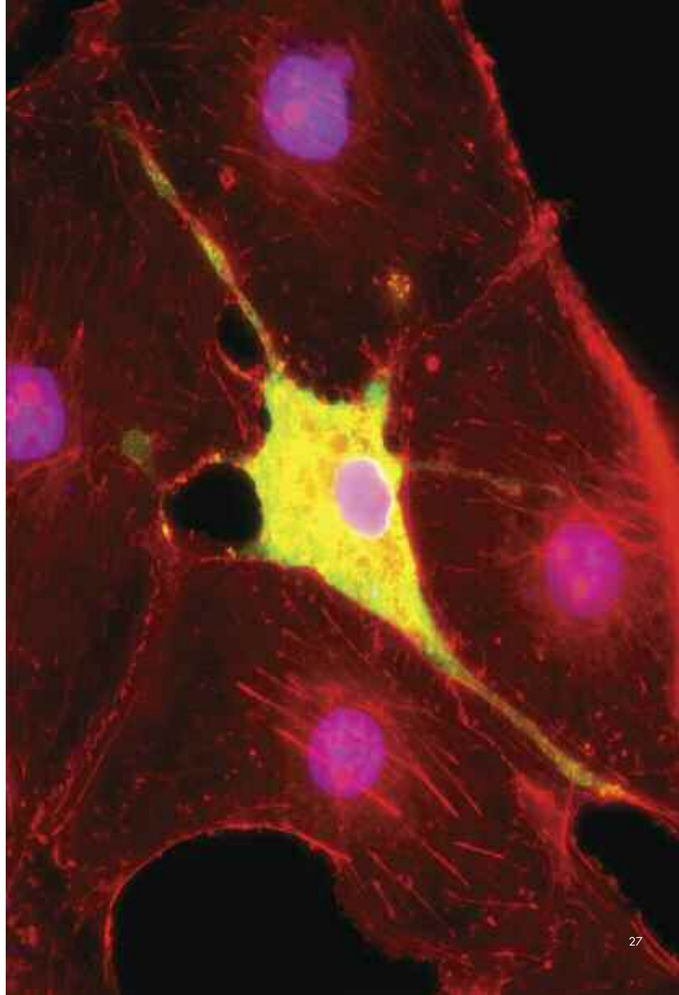
Other Professional Activities

Edward H. Hinchcliffe

Review panel, National Science Foundation, USA

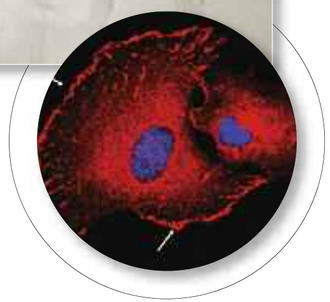
Ad hoc reviewer:
Medical Research Council, UK
Wellcome Trust, UK
Deutsche Forschungsgemeinschaft (DFG: German Research Foundation)
Agence Nationale de la Recherche (ANR: French National Research Agency)
Netherlands Organization for Scientific Research (NWO)





TUMOR MICROENVIRONMENT AND METASTASIS

Section Leader
Ningling Kang, Ph.D.
Assistant Professor



Heptic stellate cells were subjected to immunofluorescence staining for VASP (red) and cell nuclei were stained blue.

A. Summary of research directions and findings:

The liver is a preferred organ for metastasis of many malignant cancers, including gastrointestinal cancers; melanoma; breast and lung carcinomas; neuroendocrine tumors; and sarcomas. Liver metastasis remains a principal cause of patient death despite significant advances in the treatment of cancer and this metastatic liver disease.

My research program is focused on bidirectional interactions between cancer cells and the liver microenvironment critical for the development of liver metastasis. Specifically, we are interested in the interactions between cancer cells and hepatic stellate cells (HSCs), which are resident liver pericytes. Tumor-derived factors, such as TGF-beta, induce transactivation of quiescent HSCs into cancer associated fibroblasts (CAFs). In turn, CAF/activated HSCs promote tumor implantation and growth in the liver; tumor angiogenesis; and tumor chemo and radio resistance as well as suppress the anti-tumor immune responses. Furthermore, the bidirectional interactions between cancer cells and CAFs/activated HSCs may function as an "amplification loop" to further enhance metastatic tumor growth in the liver. Understanding mechanisms governing tumor/HSCs interactions is important for developing new interventions to target liver-specific mechanisms for preventing and treating tumors that have a predilection for liver metastases.

During the past year, we identified two critical factors that mediate myofibroblastic activation of HSCs under TGF-beta stimulation, which may present therapeutic targets for inhibiting tumor/HSCs interactions, reducing tumor implantation and metastatic growth in the liver. Our studies' main findings are summarized below:

1. Vasodilator stimulated phosphoprotein (VASP) of HSCs is required for recycling of TGF-beta receptors to the plasma membrane. In cell culture, VASP knockdown inhibited TGF-beta mediated activation of HSCs into myofibroblasts. VASP knockdown increased total protein levels of TGF-beta receptor II (TβRII); however, it inhibited plasma membrane protein levels of TβRII. As revealed by cell and biochemical studies, VASP formed protein complexes with TβRII and Rab11, a Ras-like small GTPase and key regulator of recycling endosomes. VASP knockdown impaired Rab11 activity and Rab11 dependent recycling of TβRII to the plasma membrane, thereby desensitizing HSCs to TGF-β1 stimulation. Conditioned medium of HSCs was collected and tested for its role in colorectal tumor cell proliferation and migration in vitro. Indeed, VASP knockdown impaired the paracrine effect of CAF/activated HSCs on tumor cell proliferation and migration in vitro. VASP knockdown HSCs also exhibited less effect on promoting colorectal tumor implantation and growth in mice as compared to control HSCs in a tumor/HSCs coimplantation mouse model. Furthermore, colorectal cancer cells induced upregulation of VASP of HSCs in an experimental metastasis mouse model and cancer patients. VASP expression levels in liver tumors correlated with poor survival of cancer patients. In summary, our data highlight the bidirectional interactions between tumor cells and HSCs for liver metastases and VASP/Rab11 complexes of HSCs as a target for inhibiting the activation of the hepatic tumor microenvironment.

2. PDGF receptor alpha (PDGFRα) promotes HSC activation via transcriptional and post-transcriptional regulation of TGF-β receptors. HSCs express both PDGFRα and PDGFRβ receptors. Knockdown of PDGFRα but not PDGFRβ, however, inhibited TGF-β induced phosphorylation and nuclear accumulation of SMAD2. As revealed by real-time RT-PCR, PDGFRα knockdown suppressed TGF-beta receptor I (TβRI) but increased TβRII gene transcription. At the protein level, PDGFRα was recruited to TβRI/TβRII complexes by TGF-β stimulation. PDGFRα knockdown blocked TGF-β mediated internalization of TβRII and subsequently induced accumulation of TβRII at the plasma membrane, thereby inhibiting TGF-β downstream signaling. Functionally, knockdown of PDGFRα reduced paracrine effects of HSCs on colorectal cancer cell proliferation and migration in vitro as well as in mice and patients. Colorectal cancer cell invasion of the liver induced upregulation of PDGFRα of HSCs. Taken together, our finding highlights a convergence of PDGF and TGF-β signaling for HSC activation and PDGFRα of HSCs as a therapeutic target for liver metastasis and other settings of HSC activation.

> "Liver metastasis remains a principal cause of patient death despite significant advances in the treatment of cancer and this metastatic liver disease."
>
> *Dr. Ningling Kang*

B. Other professional activities

Invited seminars/presentations

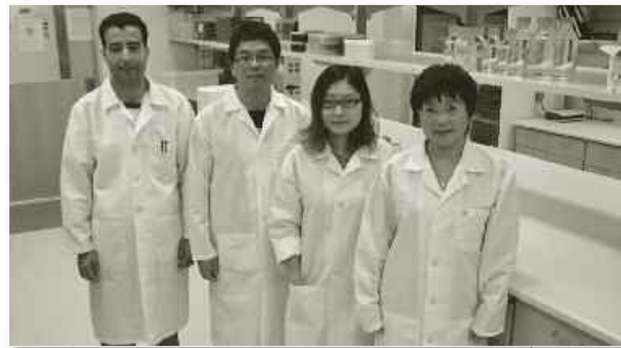
1. GI Seminar Series, Basic GI Research Unit, Mayo Clinic
2. Joint Cell Biology Group Meeting, C-SiG/GI Cancer Program, Mayo Clinic

Poster presentations

1. AASLD, The Liver Meeting, Nov. 1-5, 2013, Washington, DC
2. AACR Special Conference, Cellular Heterogeneity in the Tumor Microenvironment, Feb. 26-Mar. 1, 2014, San Diego, CA

Journal Reviewer

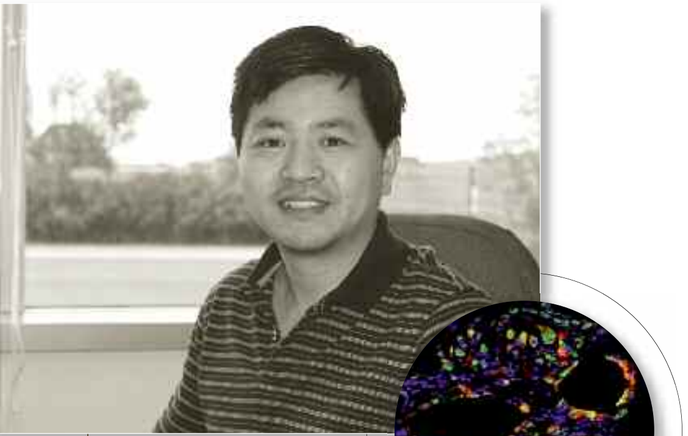
1. Journal of Hepatology
2. Molecular Cancer
3. Molecular and Cellular Biochemistry



Left to right: Ahmed Chahdi, Xiaoyu Xiang, Luyang Guo, Ningling Kang
Not pictured: Jiachu Li, Yali Xu

IMMUNOREGULATION OF AUTOIMMUNE DISEASES AND CANCER

Section Leader
Bing Li, Ph.D.
Assistant Professor



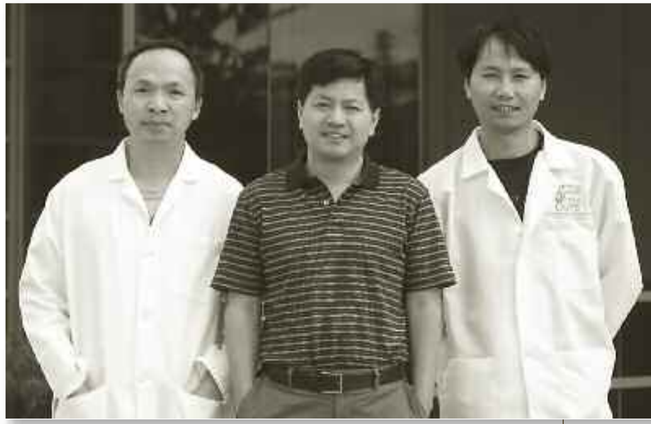
A-FABP (red color) is abundantly expressed in tumor infiltrated macrophages (green color) in breast cancer.

The main focus of this section is to understand the role of fatty acid binding proteins (FABPs) in autoimmune diseases and cancer development. FABPs constitute a family of small, highly homologous intracellular lipid chaperones that have been recognized as central regulators of both metabolic and inflammatory pathways. We have shown that adipose FABP (A-FABP) and epidermal FABP (E-FABP) play important roles in autoimmune diseases, such as experimental autoimmune encephalomyelitis (EAE) model, a mouse model of human multiple sclerosis (MS), and in different types of cancer, including breast, skin and colon cancers. The exact mechanisms underlying these observations, however, remain undetermined. My laboratory's research currently strives to understand how FABPs regulate cellular metabolism and intracellular signal transduction pathways in leukocytes; determine the mechanisms by which FABPs link metabolism and complex diseases; and identify specific inhibitors of FABPs for potential drug discovery.

For the immunoregulation of autoimmune diseases, we are focusing on the EAE model to dissect how E-FABP regulates leukocyte differentiation and functions, and to development of E-FABP inhibitors for treatment of EAE. MS continues to be a serious public health problem without a curative treatment. Although the development of MS is attributed to a combination of genetic and environmental factors, the exact cause of MS is not completely understood. The strategies of MS therapy are to modify the disease course and manage symptoms. Identification of new targets for MS prevention and management, therefore, are urgently needed. This project aims to identify E-FABP as a new risk factor for MS and produce mechanistic insights into modification of EFABP activity for MS management. As lipid chaperones, FABPs can regulate cellular metabolism and function through enabling fatty acid distribution and coordinating their responses. With fatty acids functioning as both energy sources and signaling molecules, FABPs have been identified as central regulators of metabolic and inflammatory pathways. In our previous studies, we have shown that E-FABP deficiency significantly protects mice against the development of EAE, a mouse model of MS. Mechanistically, E-FABP regulates the function of both antigen presenting cells and autoreactive T cell subsets. E-FABP deficient macrophages and dendritic cells, for example, are defective in producing proinflammatory cytokines and inefficient in antigen presentation. EFABP, but not other FABPs, is abundantly expressed in a type of T lymphocytes, which can drive pathogenic T cell differentiation to MS. Furthermore, we have demonstrated that E-FABP can counter-regulate regulatory T cell differentiation through regulation of lipid-mediated pathway. E-FABP-deficient T cells show enhanced PPARγ expression and reduced expression of RORγt and RORα. We currently have identified several promising small molecules that can ameliorate the symptoms of EAE through modulating EFABP/PPAR pathways. Thus, E-FABP offers a new target for MS pathogenesis, and targeting E-FABP might represent a novel strategy for MS and other inflammatory diseases.

For the immunoregulation of cancer development, we are focusing on mouse models of breast cancer. Each year, breast cancer kills nearly a half-million women, of whom about 90 percent die from distant metastases. Uncovering new mechanisms of breast cancer development and identifying anti-metastatic agents, therefore, represent critically important foci for prevention and/or treatment of breast cancer. In addition to such underlying risk factors as genetic background, family and reproductive history and aging per se, epidemiological studies have identified that obesity is associated with poor prognosis and increased mortality in women with breast cancer. Given the rising rates of obesity in the United States and worldwide, there is an urgent need to identify biological mediator(s) that can link obesity, immunosurveillance, and breast cancer development. The goal of this research project is to identify new mediators and produce mechanistic insights into the biology of their action in mediating breast cancer progression and metastasis. Carcinogenesis is a multistep, complex process that depends on the crosstalk between cancer cell intrinsic factors and extrinsic immunosurveillance effects. Obesity has been established as a risk factor for cancer incidence and cancer mortality. It is likely that obesity may contribute to cancer development either through promotion of cancer cell intrinsic transformation or through subversion of the extrinsic host immune system. Our preliminary studies have demonstrated that crosstalk of tumor cells and tumor infiltrating macrophages can significantly stimulate the cytoplasmic expression of A-FABP in macrophages, which switches the phenotype of macrophages to pro-tumor development. Strikingly, a strain of mice that lacks A-FABP has been found to be protected from breast cancer development. While FABPs traditionally are recognized as cytoplasmic lipid chaperones enabling lipid distribution and coordinating their responses inside cells, we have found that obesity can significantly upregulate A-FABP expression in both macrophages and adipocytes. More importantly, tumor associated adipocytes can secrete a large amount of A-FABP into circulation in response to exposure to tumors, which may serve as a plasma biomarker for early cancer diagnosis. Our studies, therefore, suggest that A-FABP exerts its effects through both local (cytoplasmic) and systemic (circulating) actions. A-FABP may promote carcinogenesis and tumor development by targeting both cancer cells and immune cells and offers a new therapeutic target for cancer treatment. In this manner, A-FABP may represent a new link between obesity and cancer.

> "Given the rising rates of obesity in the United States and worldwide, there is an urgent need to identify biological mediator(s) that can link obesity, immunosurveillance, and breast cancer development."
>
> *Dr. Bing Li*

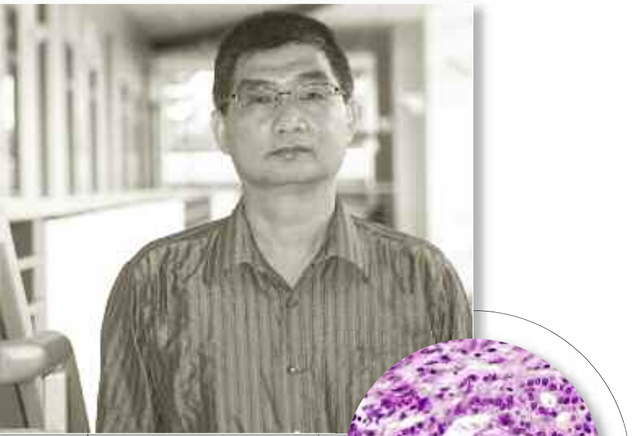


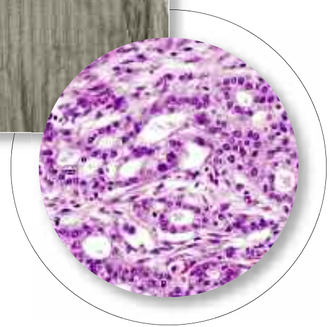
Left to right: Yuwen Zhang, Bing Li, Enyu Rao
Not pictured: Qiang Li

In summary, our studies have revealed that FABPs play essential roles in regulating cellular metabolism and immune functions. While A-FABP is more critical in regulating functions of macrophages and adipocytes in tumor development, E-FABP exhibits a unique role in T cell differentiation in inflammatory autoimmune diseases. These results will have significant implications in their potential applications.

TRANSLATIONAL CANCER RESEARCH

Section Leader
D. Joshua Liao, M.D., Ph.D.
Associate Professor





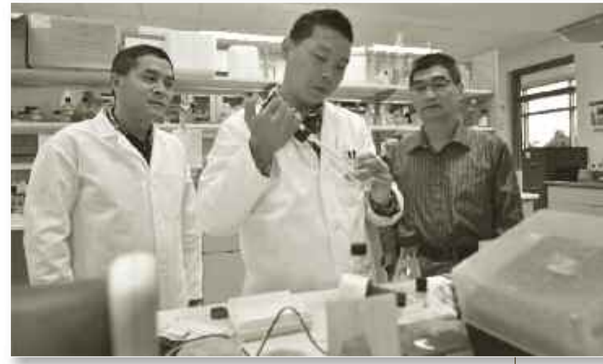
Micrograph of pancreatic ductal adenocarinoma (the most common type of pancreatic cancer).

In the past year, we continue working on a U.S. Department of Defense (DOD) funded project that aims to identify fusion RNAs as possible biomarkers for breast cancer. Our results achieved so far lead to a conclusion that the vast majority of over a million of putative fusion RNAs documented in the literature may be technical artifacts. In a paper we published recently, we propose major technical reasons for the possible creation of the artifacts. Of those truly existing fusion RNAs, most are associated with a corresponding fusion gene in the genome. In breast cancer, however, basically all fusion RNAs are not recurrent, and this feature emphasizes the importance of personalized diagnosis and treatment. Of some fusion RNAs that occur at the RNA level without a genomic basis, mitochondrial RNAs may participate in their formation. In other words, human mitochondrial RNAs also undergo cis- and trans-splicing and fuse with nuclear RNAs to enlarge the cellular RNA repertoire, which implies a previously unaware mechanism for RNA fusion that may occur at the cytoplasm, but not in the nucleus.

Many of the fusion RNAs may be translated to proteins that differ from their wild-type protein in molecular weights. To test this hypothetical thinking, sophisticated LC-MS/MS technique for the first time is used to stratify proteins by their molecular weight from narrow stripes of the SDS-PAGE gel. The results show that only about one-fourth (24.5%) to one-third (36.2%) of the LC-MS/MS identified proteins have a molecular weight as calculated from the wild-type protein sequence while 8.4% to 26.0%, varying among cell types, of the proteins have a larger molecular weight, in part because they are a larger isoform. Most of the remaining proteins (varying between 67.1 to 37.8%) may be smaller isoforms or proteolyzed fragments, 42.1-47.5% of which – at least in one cell type – may have at least two smaller isoforms besides the wild-type protein. These startling figures – although they may vary among cell types – strongly indicate that a large number of genes produce different protein isoforms, some of which may be products of fusion RNAs. This information should be of important guidance not only to the researchers on determining protein expression but also to the antibody producers for their determination of the antibody specificity because selection of those antibodies that detect only the protein with anticipated molecular weight may be a bias in some situations.

In the past year, we also have initiated a new research project to develop a new cancer therapeutic regimen. Whole body hyperthermia (i.e. systemic increase in the body temperature) has been used clinically for decades to treat cancer, either alone or adjuvant to chemotherapy or irradiation. In almost all cases, however, the whole body temperature is raised by sophisticated warming devices to a high, feverish range for only a short period (a few hours). In addition, there are many attempts aiming to develop chemical inhibitors of heat-shock proteins (HSPs) as anticancer drugs because most cancers manifest elevated levels of HSPs to survive not only heat but also many other forms of stress. Besides, hyperthermia can enhance the innate immunity against cancer cells. We hypothesize that combined hyperthermia, HSP inhibition and chemotherapy, abbreviated as HHIC, may be a better regimen for cancer treatment because cancer cells no longer can further raise their HSP levels during hyperthermia while normal cells have low basal levels of – and, thus, still can raise – HSPs for cytoprotection. Our preliminary studies on culture cancer cells show promising results that a feverish temperature (39 ºC) can enhance the killing effects of several chemo drugs on different cancer cell lines. Moreover, KRIBB11, an inhibitor of heat shock factor-1 that is a master activator of many HSPs, also enhances chemotherapeutic effects, especially at a feverish (39 ºC) temperature. These results lead us to the proposal of HHIC as a better cancer treatment regimen, as illustrated in the figure.

> "In the past year, we also have initiated a new research project to develop a new cancer therapeutic regimen."
>
> *Dr. D. Joshua Liao*



Left to right: Jiangang Wang, Shengming Zhu, Joshua Liao
Not pictured: Wu Chen, Yongchang Ouyang

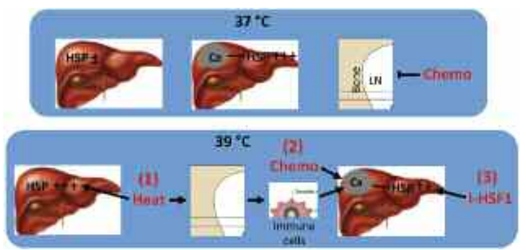
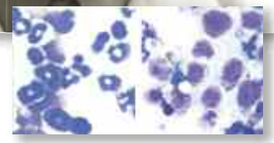


Figure legend: Illustration of combined hyperthermia, heat-shock protein (HSP) inhibition by heat shock factor-1 inhibitor, and chemotherapy as a novel cancer treatment regimen using liver cancer as an example. At a normal situation (top panel), many HSPs already are overexpressed (+++) in cancers; whereas, chemo drugs usually inhibit immunity, such as inhibition of cells in bone marrow or lymph nodes (LN). Our novel regimen includes three elements: i.e. (1) the elevated body temperature that not only activates immune cells but also induces HSPs in, and, thus, to protect, normal cells; (2) a standard chemo or radiation therapy; and (3) a systemic treatment with a heat-shock protein inhibitor, such as an HSF1 inhibitor.

CANCER EPIGENETICS & EXPERIMENTAL THERAPEUTICS

Section Leader
Shujun Liu, Ph.D.
Associate Professor



Giemsa-stained bone marrow cells from leukemia-bearing lean (left) and obese (right) mice.

Primary interests of our research section are to understand the molecular mechanisms and roles of aberrant epigenetics and protein-kinase activity in cancer pathogenesis. We also aim 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 disease sites. In our laboratory, studies have included the cause of DNA hypermethylation and abnormal protein-kinase activity; the mechanistic link between obesity and leukemia; the dissection of molecular basis underlying the anti-cancer actions of bioactive compounds; and the development of innovative nanoparticulates for drug delivery.

The molecular rules underlying aberrant epigenetics

DNA methylation occurs at the 5-position of cytosine in a CpG dinucleotide context and is a major epigenetic mechanism regulating chromosomal stability and gene expression. DNA methylation is under control of DNA methyltransferases (DNMTs), including DNMT1, DNMT3a and DNMT3b. In cancers, DNMTs are highly expressed and the tumor-suppressor genes (TSGs) frequently are silenced by promoter hypermethylation. The molecular processes behind DNMT overexpression, however, remain largely unclear. We have demonstrated that Sp1/miR29 feedback loop critically regulates the expression of DNMT1, DNMT3a and DNMT3b in leukemia. Pharmacological modulation of Sp1/miR29 network by siRNA and/or small molecules changes DNA methylation status leading to the reexpression of TSGs and the inhibition of leukemia growth. Since Sp1 and miR29b target DNA methylation at multiple levels, we demonstrated that the combination of Sp1 inhibitors (siRNA or bortezomib) with synthetic miR29b synergistically suppresses their downstream signaling cascades. In addition, we showed that miR101 directly suppresses DNMT3a expression leading to DNA hypomethylation. We presented evidence that nucleolin upregulates DNMT1 through hyperactive NFkB signaling pathway, and nucleolin inhibitor AS1411 displays DNA hypomethylating activity. All of these observations point out the complexity of DNA methylation machinery, which may explain – at least in part – why current epigenetic therapies in cancers have disappointing results.

The molecular mechanisms controlling the transcription of protein kinases

It is well known that abnormal kinase activities are essential in cancer initiation and metastasis, but the main focus currently is to pharmacologically inhibit kinase activities without the consideration of kinase overamplification. We, along with others, have demonstrated that kinase gene overexpression is involved in the development, progression and drug resistance of cancer as well as in survival in some patient subpopulations. How protein kinases are transcriptionally regulated, however, remains incompletely understood. We previously reported that receptor tyrosine kinases (i.e., KIT and FLT3) are regulated by Sp1/miR29 network. Given the critical roles of Sp1/miR29 in DNMT gene regulation, we proposed that aberrant DNMT activities also might control kinase signaling. Indeed, we found that KIT and DNMT1 are highly elevated, and their expression is positively correlated in lung cancer. We demonstrated that DNMT upregulation increases KIT expression, leading to KIT specific and global kinase over-function; in turn, enforced KIT expression enhances DNMT1 gene levels and induces global and gene specific DNA hypermethylation. Importantly, co-knockdown of KIT and DNMT1 synergistically impairs the metastatic growth of cancer cells in lungs. These findings identify the regulatory and functional interactions between kinases and DNA methyltransferases, and suggest that misregulated KIT signaling and DNA hypermethylation cooperatively contribute to cancerous lesions.

Mechanistic links between obesity and leukemia

Cancer is the representatively systemic lesions taking over the first place of lethal diseases throughout the world. Obesity is a "disease" with abnormal body fat accumulation. The World Health Organization estimates that approximately one quarter of the world's population is obese. Emerging data indicate that obesity is a major risk factor for human malignancies. It can increase the occurrence of cancerous lesions and decrease the benefit of therapy. The molecular mechanisms behind these phenomena, however, are poorly defined. We observed that higher body mass index (BMI) associates with shorter overall survival in leukemia patients. When leukemia cells were transplanted into obese or lean mice, we found that – compared to the lean counterparts – obese mice display exacerbated leukemic disease, thus, experimentally demonstrating the contribution of obesity to leukemogenesis in mice. Mechanistically, a family of fatty acid binding protein (FABPs) could mediate obesity associated leukemia. Leukemia cells, in fact, abundantly express FABP4 (aP2) that is highly expressed in obesity. Treatment with FABP4 recombinant protein accelerates leukemia cell growth. The future directions are to delineate the molecular pathways controlling the FABP4-induced aggressive leukemia growth.

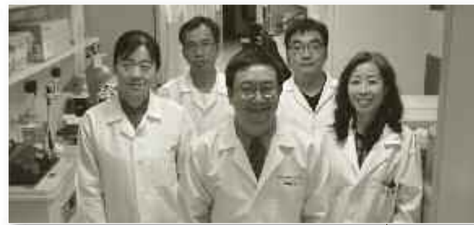
> "We observed that higher body mass index (BMI) associates with shorter overall survival in leukemia patients."
>
> *Dr. Shujun Liu*

Molecular mechanisms of anti-cancer actions of bioactive compounds

Due to the essential roles of aberrant DNA methylation in cancers, DNA hypomethylating agents have been in clinical use for decades. While some positive response has been achieved, the majority of leukemia patients relapse and eventually die of their disease, arguing for the new type of DNMT inhibitors with different structure and distinct mechanisms. Because of their anti-cancer activity and lower toxicity to normal cells, numerous plant extracts have been tested in vitro and in vivo with huge therapeutic potentials. We have demonstrated that certain types of bioactive compounds (i.e., thymoquinone (TQ), echinomycin or emetine) suppress the expression of DNMT1, DNMT3a and DNMT3b, resulting in global DNA hypomethylation and the re-expression of TSGs by promoter hypomethylation. Functionally, these compounds block cancer cell proliferation in cell lines, patient primary cells and metastatic growth in vivo. While the underlying molecular mechanisms remain elusive, these compounds may hold a promising future in human cancer therapy.

Developing multifunctional drug and gene delivery nanoparticles for cancer therapy

The current chemotherapeutic drugs (i.e., small molecules, siRNA or miRs) – although they display promising anti-cancer activity – suffer from a variety of drawbacks when administered particularly in vivo, including rapid clearance, lack of tissue selectivity, high affinity to plasma proteins and poor cellular uptake. We have developed new liposomal formulations and synthesized nanoparticles to efficiently deliver the aforementioned drugs. We demonstrated the synergy between bortezomib and miR29b, which were delivered by liposomal nanoparticles in promoting DNA hypomethylation in vitro. We have successfully delivered bortezomib, miR29 and Sp1 siRNA by nanoparticles in vivo. As a consequence of efficient delivery, we observed that liposomal bortezomib has a decrease of clearance and, thereby, an increase of drug exposure to leukemia cells existing in blood compared to those of free bortezomib in mice. We also evidenced the synergistic effects of combined liposomal bortezomib with nano-miR29b on leukemia cell growth in mice. Recently, we synthesized HDL/AuNP nanoparticle and successfully delivered small molecule compounds into leukemia cell lines, patient primary cells and in leukemic mice, which was demonstrated by the inhibition of leukemia cell colony formation, reduction of DNA methylation and blockage of leukemia growth in mice. These results revealed that nano-drug delivery displays huge potential to improve therapeutic efficacy while reducing its side effects, including decreased drug toxicity, altered pharmacokinetics, improved drug solubility and more specific target binding.

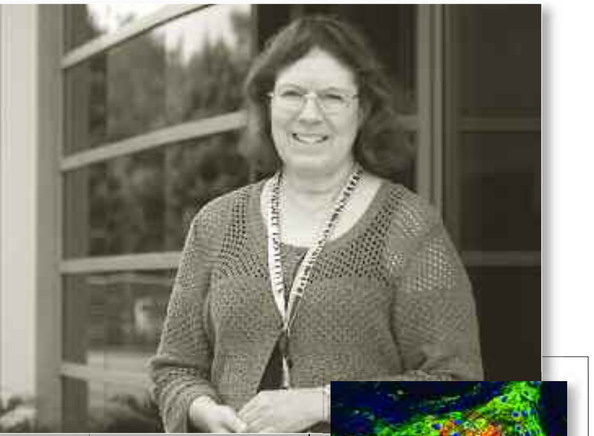


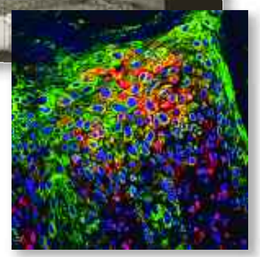
Left to right: Liping Dou, Shengcai Wei, Shujun Liu, Fei Yan, Jiuxia Pang
Not pictured: Na Shen, Xiao-ning Gao

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

STEM CELLS AND CANCER

Section Leader
Rebecca J. Morris, Ph.D.
Professor





Skin keratinocytes

The Morris laboratory studies the role of stem cells in cancer. We focus on normal adult stem cells responsible for maintenance, replacement, and regeneration of all of the body's tissues. In particular, we study adult stem cells from hair follicles, mammary glands, and bone marrow. We recently demonstrated that stem cells from hair follicles and bone marrow both contribute to the pathogenesis of nonmelanoma skin cancer. Ongoing studies are focused on the cellular and molecular mechanisms of stem cell regulation, and how bone marrow-derived cells interact with epithelial cells in skin and mammary gland in the development of cancer. This work will aid in the identification of novel targets for cancer prevention, diagnosis, and therapy.

Most of our studies involve the epidermis. The skin's epidermal layer is composed largely of cells called "keratinocytes." Keratinocytes in the basal layer are organized into subpopulations based on their proliferative nature and include stem cells (relatively rare) and transit-amplifying cells (comprise most of the proliferating cells). When a stem cell divides, one daughter usually remains a stem cell while the other daughter gives rise to transit-amplifying cells with limited proliferative potential. Upon completion of their divisions, transit-amplifying cells undergo an orderly maturation process called "terminal differentiation" that includes their outward displacement through the suprabasal layers, production of high-molecular-weight keratins, loss of their nuclei, and formation of an impervious outer structure called the "cornified envelope." This process is exceptionally orderly and maintains the normal thickness and cellularity as well as the normal functions of the epidermis throughout life. We have focused on the stem cells of the hair follicles because they not only serve as a reservoir of epidermal cells but also possess remarkable regenerative potential. They also are known to be able to reconstitute a graft, heal wounds, and even give rise to non-melanoma skin cancer. Identification of stem cell behavioral characteristics and responses, therefore, are critical problems in cutaneous biology. The Morris laboratory is focusing on two, specific projects.

The first project is related to the interactions between epithelial cells and cells of the bone marrow. We are employing both in vitro models of co-culture and migration and in vivo models, using transplantation of genetically labeled bone marrow. Although these experiments are still in progress, we have found evidence of a dynamic interaction between the epidermis and bone marrow-derived cells in vitro and in vivo. We now are working on the mechanism of these interactions. To this end, we documented the recruitment of bone marrow-derived epithelial cells to a subset of cutaneous papillomas.

Bone marrow-derived epithelial cells are known to be recruited to sites of chronic inflammation; however, their clinical significance has been questioned. To address this problem, we used allogeneic bone marrow transplantation in the context of the classical multistage model for murine cutaneous carcinogenesis to probe the recruitment of bone marrow-derived epithelial cells in skin tumors initiated with the carcinogen 7,12 dimethylbenz[a]anthracene, and promoted with the phorbol ester, 12-O-tetradecanolyphorbol-13-acetate. We detected clusters of bone marrow-derived epithelial cells in more than 40 percent of papillomas, where they occupied 25 percent or more of the lesional area. In dysplastic ulcers, the magnitude of the recruitment was greater. The bone marrow-derived epithelial cells clustered in the cutaneous epithelium, where they became immunoreactive to epidermal keratins as well as proliferated and stratified, thereby contributing comparably in the lesions with the progeny of hair follicle stem cells in engrafted Krt1-15Cre;R26R mice. Moreover, cytokeratins were detected by immunostaining and quantitative, real-time polymerase chain reaction in plastic, adherent bone marrow cells cultured in the presence of filter-separated epidermal keratinocytes. Cytokeratin production was enhanced in vitro by bone morphogenetic protein 5. We conclude that large numbers of bone marrow-derived epithelial cells are recruited to a subset of cutaneous papillomas and dysplastic ulcers and reflect a previously unrecognized systemic contribution to these lesions. Ultimately, these findings may provide potential targets for treatment of non-melanoma skin cancers.

> "Ultimately, these findings may provide potential targets for treatment of non-melanoma skin cancers."
>
> *Dr. Rebecca J. Morris*

In the second project, we are working on the identification of keratinocyte stem cell regulatory genes. Keratinocyte stem cells have an unquestioned role in maintaining the normal structure and function of the epidermis and hair follicles and are thought to be important players in inherited and acquired skin diseases. Hence, identification of genes regulating their number and proliferative potential is a critical problem in cutaneous biology. To address this problem, we proposed a novel strategy for identifying genes involved in keratinocyte stem cell regulation. This strategy takes advantage of several important advances made in our laboratory: 1) identification of selectable markers on hair follicle stem cells; 2) development of a sensitive and quantitative in vitro assay for clonogenic keratinocyte stem cells; 3) genetic mapping of several loci with linkage to stem cell number; and 4) analysis of keratinocyte stem cell gene expression. The objective is to identify major genes regulating the number of keratinocyte stem cells. Our hypothesis is that there are specific genes and pathways that regulate stem cells that may be different from those regulating transit-amplifying cells. First, we are using genetic tools to refine the linkage intervals we already have identified. Second, we are using a candidate-gene approach for stem-cell gene identification. Third, we are using a complementary, global-genomic approach to identify associated molecular pathways and assess regulatory polymorphisms causing differences in gene expression in the absence of differences in coding sequences within the QTL. This research will affect the fields of cutaneous biology and stem cell research and should provide new insights into the mechanisms of cutaneous carcinogenesis. Identification of stem cell-regulatory genes is important for gene therapy as well as for the design of new, therapeutic modalities for chronic hyperproliferative skin disease; wounds that do not heal; and skin cancer.

In summary, research in the Morris laboratory continues to highlight the role of hair follicle stem cells in the pathogenesis of non-melanoma skin cancer, and has documented an unexpected contribution of bone marrow-derived cells. Going forward, we will continue to investigate the regulation of skin stem cells in cancer as well as the interactions between epidermal stem cells and bone marrow-derived cells as tumor-initiating and -propagating cells.

Left to right: Rebecca Morris, Kelsey Boland, Mi Sung Kim, Kelly Johnson, Yong Li.
Not pictured: Nyssa Readio

RESEARCH SUPPORT GROUP

Supervisor
Ellen Kroc

The Hormel Institute's Research Support Group (RSG), supervised by Ellen Kroc, provides vital operational support within the Institute's 13 research sections for their many ongoing research projects. Each of the Institute's cancer research departments is dedicated to preventing or controlling cancer.

Left to right: Ellen Kroc, Michelle Jacobson, Melissa Fortsch, Teri Johnson
Not pictured: Sherri King, Zach Soiney

LIBRARIAN

Andy Lucas

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 16th largest in North America by collection size. Books are delivered through the MINITEX delivery service and are available for pickup in The Hormel Institute Library. Articles that are not available in electronic form are obtained through interlibrary loan.

INSTRUMENT CORE FACILITY

Todd Schuster
Senior Lab Technician

Schuster operates, maintains and instructs scientists about the shared instruments used at The Hormel Institute for cancer research. Shared instruments and equipment include: Becton Dickinson FACS Aria II cell sorter, FACSCalibur flow cytometer, ABSCIEX 5600 Triple TOF mass spectrometer and Eksigent NanoLC nano HPLC system, Rigaku X-Ray diffraction system for protein crystallography, confocal and fluorescent microscopes, real time PCR, spectrophotometers, tissue processor and microtome, cryostat, and high speed and ultracentrifuges.

OFFICE

Supervisor
Associate Director
Ann M. Bode, Ph.D.

Our office staff continues to provide excellent editorial and clerical support to the research sections and serves as liaison with the University's central administration departments. Each year, staff members travel to the Twin Cities campus to participate in refresher training and various workshops relevant to their duties.

Left to right: Dr. Ann M. Bode, Jessica Swanson, Betsy Mentel, Nicki Brickman
Not pictured: Cheryl Corey, Constance Post

PARTNERS IN GROWTH

PUBLIC RELATIONS AND DEVELOPMENT

Director
Gail Dennison, M.A., CFRE

Our guiding principle is to win support for The Hormel Institute's quest to improve the health of the world. Our focused team of expert researchers aim daily to discover the mechanisms of cancer – what will prevent it and what are healthier paths to control it.

In 2013-2014, more individuals, businesses and organizations stepped forward than ever before to support The Hormel Institute's groundbreaking cancer research. The visionary support of The Hormel Foundation, led by Chairman Gary Ray, places The Hormel Institute on a path where the future is truly limitless for what can only be called TRANSFORMATIVE CHANGE.

Our friends and collaborators know and understand the unique story of The Hormel Institute. Together we know for a healthier tomorrow, research must be funded today.

Thank you, one and all, for sharing our vision of "Today's Research, Tomorrow's Cures."

Left to right: Gretchen Ramlo, Tucker Mithuen, Gail Dennison, Tim Ruzek, Michelle Phillips
Not pictured: Kelsey Stough (intern)

"The Eagles Cancer Telethon recognizes that The Hormel Institute is on the leading edge of cancer research, and we are proud to support them in their work and continued fight against cancer."

--- Teresa Chapman,
Director, 5th District Eagles Cancer Telethon

coming together for the cure

Thank You for furthering Cancer Research

The Hormel Foundation
Hormel Foods Corporation
Mayo Clinic Health System
Minnesota Governor Mark Dayton
U.S. Senator Amy Klobuchar
U.S. Senator Al Franken
U.S. Representative Tim Walz
State Senator Dan Sparks
State Senator David Senjem
State Representative Jeanne Poppe
Mayor of Austin – Tom Stiehm
Mayor of Rochester – Ardell Brede
Dr. Harald & Pat Schmid Endowed Professorship
Richard & Nancy Knowlton
Gary & Pat Ray
5th District Eagles Cancer Telethon
Lyle Area Cancer
U.S. Bank
Ecolab
Estate of Carol L. Landgraf
Austin Bruins "Paint the Rink Pink"
"Paint the Town Pink" Austin, Brownsdale, Rose Creek
Karl R. Potach Foundation
Minnesota VFW Ladies Auxiliary
AgStar Fund for Rural America
Minnesota Department of Employment & Economic Development
Austin Area Chamber of Commerce
Austin Area Foundation
Austin Convention & Visitors Bureau
BioBusiness Alliance of Minnesota
City of Austin – Austin Port Authority
GRAUC – Greater Rochester Advocates of Universities and Colleges
IBM Rochester
University of Minnesota – Rochester
Mower County
Riverland Community College
Austin Public Schools
Pacelli Catholic Schools
Southern Minnesota Initiative Foundation
Dave "Tolly" Tollefson Memorial Golf Tournament
Deryl Arnold Memorial Golf Tournament
Fishing for a Cure
Mower County USBC Association's "Bowl for a Cure"
The Hormel Institute Mentor Group
Mower County Fair Board
YMCA of Austin
Austin Vision 2020
Norma Foster Memorial "Ride for a Reason"
Jim & Vicky King/Spiritually Motivated
St. Olaf Dining Committee
KAUS "Pledge for Pink" radiothon
Blooming Prairie Cancer Group
Pheasants Forever "Hunt for the Cure"
St. Marks Lutheran Home
Hormel Historic Home
Austin ArtWorks Festival

Today's RESEARCH,
Tomorrow's CURES

inspire
unite

hope
believe

DR. HARALD & PAT SCHMID PROFESSORSHIP ENDOWMENT

Dr. Harald Schmid, who joined the Institute in 1962 and served as executive director from 1987 to 2001, gave $1 million for an endowed professorship from him and his wife, Pat. Schmid retired in 2004.

Left to right: Theresa Tucker, Craig Jones, Tim Lastine, Mike Conway

RESEARCH SUPPORT SERVICES (RSS)
Supervisor: Craig Jones

It has been another exciting year for RSS. The Linux cluster CAL42 (Computational Analysis of Life-sciences 42) has been calculating away, simulating protein molecules.

Research Support Services continues to provide instrument maintenance as well as computer, graphics, 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 workload. As always, the security needs of the network keep us busy.

Plans are progressing extremely well for our $27 million expansion project supported by The Hormel Foundation, Austin Port Authority and the State of Minnesota. We are getting real excited about moving some dirt and actually getting started building. There will be 20 new laboratories to set up and all the new instrumentation that goes with them. It is scheduled to be completed by fall of 2015, so we will have a lot of infrastructure to get ready. This has been another great year for us, and next year is looking to be even more exciting.

BUILDING OPERATIONS AND MAINTENANCE
Supervisor: Mark Severtson

Left to right:Randy Johnson, Brandon Hoium, Duane Graff, Mark Severtson

The maintenance support unit's main goal is to provide all personnel with a comfortable and safe working environment. Regular inspection and maintenance of all buildings and equipment is performed to assure continuous, efficient operation and comfort. All safety equipment is routinely checked to assure proper operation in the event of an emergency. This unit also is responsible for the receiving, recording and delivering of all incoming supplies and equipment delivered to The Institute.

Also occasional minor laboratory and office rearrangement is done to maximize efficient use of space.

This unit has regular contact with University building and safety officials to be certain that various building alterations, repairs and functions are completed according to required code and safety regulations. Local professional tradesmen are also contacted for minor repairs or alterations necessary to keep operations running safely, smoothly and efficiently within the facility. The Building Operations and Maintenance department is very proud of our new addition and remodeled facility. We will all strive to keep it looking and operating with the upmost of efficiency.

S.U.R.E. (Summer Undergraduate Research Experience)

Each year, undergraduate students are selected to work in the Summer Undergraduate Research Experience (SURE) program with The Hormel Institute scientists. Students work on research projects to expand their knowledge of basic research and learn about equipment and techniques that are not generally available in undergraduate academic programs. Each year, students are selected based on their high level of academic achievement as well as their plans to pursue careers in science-related fields.

(Left to right) Front row: Kelly Gray, Andrew Dennison, Eli Min, William Cao, Kira Potach
Middle row: David Koser, Nichole Clemens, Michael Brickman
Back row: Kevin Brockman, Brian Kurtz, Ryan Pewowaruk

Gary Ray,
Chair of The Hormel Foundation
"In just the last 10 years, The Hormel Foundation has invested $70 million into this Institute. We believe in what it's doing to beat cancer. The work of the Institute has benefited all of us here today. The real excitement, though, is going to be about the future that it brings to generations of families. The Institute is truly living up to the promise of its slogan, 'Today's Research, Tomorrow's Cures.' "

Discoveries leading to the prevention and control of cancer will be accelerated now more than ever before.

On May 28, 2014, The Hormel Institute hosted more than 350 state, federal and local leaders as well as many supporters to celebrate the start of the continuing historic progress of The Hormel Institute. This next exciting chapter in the Institute's seven-decade history of helping the health of the world through cutting-edge scientific research was launched with the groundbreaking of the current expansion that will again double the size of The Hormel Institute.

The expansion bill was introduced for the 2012 Minnesota bonding bill by local legislators Senator Dan Sparks and Representative Jeanne Poppe, and supported by nearly all state legislators. Minnesota Governor Mark Dayton, U.S. Senator Amy Klobuchar, U.S. Senator Al Franken and State Senator Dave Senjem spoke during the May groundbreaking ceremony, launching this great achievement. Following an expansion in 2008 that tripled the size of The Hormel Institute, the current expansion will double the size of The Hormel Institute, adding 20 state-of-the-art laboratories and better space for The Hormel Institute's International Center of Research Technology. The Hormel Foundation and expansion leaders are providing the space to support the hire of another 120 faculty and staff and to provide a stronger path to commercialization opportunities in Austin and southern Minnesota.

Live Learning Center

A 250-seat, globally interactive learning center/ lecture hall also is planned as an additional project to the 2014-2016 expansion at The Hormel Institute.

Projected to be completed in late 2015 / early 2016, the expansion is paid for by $13.5 million in bonding funds from the State of Minnesota (an Austin Port Authority project) and $23 million from The Hormel Foundation to support construction, technology and the hiring of faculty. Fundraising continues for the $4.5 million Live Learning Center.

Dr. Zigang Dong,
Executive Director of The Hormel Institute,
University of Minnesota
"We know today's just the beginning of the growth, of the success and also for more cancer research because we know the best way for us to say 'thank you' is to do more research, to do better research, to find a cure or prevention way of the deadly disease that is human cancer."

coming together for the cure

Minnesota Governor Mark Dayton
"This is truly the beginning … the result is doubling the size of this Institute, almost doubling the employment here. These are going to be great jobs."

Dr. Brian Herman, University of Minnesota's Vice President for Research
"Every day, this Institute makes good on its charge and helps sow the seeds of innovation around the globe. Here at the Institute, the quality of research is superb. The scientists engage in thoughtful and far-reaching study, stimulated by unique, cross-disciplinary partnerships, many with industry."

U.S. Representative Tim Walz
"The Institute is one of the shining stars in medical research. I'm certainly honored to have it in the 1st District, and proud to work as a partner with the Institute. Some of the best and brightest minds in the world working towards a cure for cancer are right in Austin."

U.S. Senator Amy Klobuchar
"This research means something. It means something to people that have cancer and can go on another month knowing that Dr. Dong and his team are fighting for them. And it means something for jobs in Minnesota in a big way."

U.S. Senator Al Franken
"This is a world leader in cancer research. And The Hormel Institute is bringing us ever closer to groundbreaking prevention and treatment therapies. Our investment in research is the key to our country's success."

State Senator David Senjem
"I'm not sure we can imagine today what might come out of these facilities but we know it's good and we know it's going to make our people better, our human race better, our world better."

State Representative Jeanne Poppe
"The Hormel Foundation's early vision and commitment to research has grown exponentially and created opportunities in all aspects of scientific discovery and research through The Hormel Institute. Expanding its footprint enhances the possibilities for discovery."

State Senator Dan Sparks
"I am proud to have been the Chief Senate Author for the state's investment of $13.5 million in the expansion project. The project gained wide support as legislators learned of The Hormel Institute's world-class reputation and groundbreaking cancer research. It is exciting to see the project underway."

The Hormel Institute Publications

July 1, 2013 — June 30, 2014

H.I. No. 1939 (Left off of FY 13)
"Novel TOPK inhibitor HI-TOPK-032 effectively suppresses colon cancer growth"
Kim DJ, Li Y, Reddy K, Lee MH, Kim MO, Cho YY, Lee SY, Kim JE, Bode AM, Dong Z. Cancer Research Jun 15;72(12):3060-8 (2012).

H.I. No. 1940 (Left off of FY 13)
"Prediction of molecular targets of cancer preventing flavonoid compounds using computational methods"
Chen H, Yao K, Nadas J, Bode AM, Malakhova M, Oi N, Li H, Lubet RA, Dong Z. PLoS One 7(5):e38261 (2012).

H.I. No. 1941 (Left off of FY 13)
"Effects of dietary effectors on signal transduction pathways related to cancer prevention"
Bode AM, Dong Z. Nutritional Genomics--The Impact of Dietary Regulation of Gene Function on Human Disease CRC 243-268 (2012).

H.I. No. 1942
"Post-processing for statistical image analysis in light microscopy."
Cardullo, R. and Hinchcliffe, E. H., (2013). Methods in Cell Biology 114: 285-315.

H.I. No. 1943
"Differential effects of genistein on prostate cancer cells depend on mutational status of the androgen receptor."
Mahmoud, A. M., Zhu, T., Parray, A., Siddique, H. R., Yang, W., Saleem, M., Bosland, M.C. (2013). PLoS ONE 8(10): e78479.

H.I. No. 1944
"65,000 shades of grey: Use of digital image files in light microscopy."
Shaw, S. L. and Hinchliffe, E. H., (2013). Methods in Cell Biology 114: 317-336.

H.I. No. 1945
"BMI1 polycomb group protein acts as a master switch for growth and death of tumor cells: regulates TCF4-transcriptional factor-induced BCL2 signaling."
Siddique, H. R., Parray, A., Tarapore, R. S., Wang, L., Mukhtar, H., Karnes, R. J., Deng, Y., Konety, B. R., Saleem, M. (2013). PLoS ONE 8(5): e60664.

H.I. No. 1946
"BMI1, stem cell factor acting as novel serum-biomarker for Caucasian and African-American prostate cancer."
Siddique, H. R., Parray, A., Zhong, W., Karnes, R. J., Bergstralh, E. J., Kuochekpour, S., Rhim, J. S., Konety, B. R., Saleem, M. (2013). PLoS ONE 8(1): e52993.

H.I. No. 1947
"Select dietary phytochemicals function as inhibitors of COX-1 but not COX-2."
Li H, Zhu F, Sun Y, Li B, Oi N, Chen H, Lubet, R. A., Bode, A. M., Dong, Z. (2013). PLoS ONE 8(10): e76452.

H.I. No. 1948
"The role of necroptosis, an alternative form of cell death, in cancer therapy."
Yu X, Deng Q, Bode AM, Dong Z, Cao Y. (2013). Expert Review of Anticancer Therapy 13(7): 883-893.

H.I. No. 1949
"USP8 is a novel target for overcoming gefitinib resistance in lung cancer."
Byun, S., Lee, S. Y., Lee, J., Jeong, C. H., Farrand, L., Lim, S., Reddy, K., Kim, J. Y., Lee, M. H., Lee, H. J., Bode, A. M., Won Lee, K., Dong, Z. (2013). Clin Cancer Res 19(14): 3894-3904.

H.I. No. 1950
"Tumor suppressor p16 INK4a inhibits cancer cell growth by downregulating eEF1A2 through a direct interaction."
Lee, M-H., Choi, B Y, Cho, Y-Y, Lee, S-Y, Huang, Z, Kundu, J K, Kim, M O, Kim, D J, Bode, A. M., Surh, Y-J, Dong, Z. (2013). Journal of Cell Science 126(16): 3796.

H.I. No. 1951
"Strategies for identifying molecular targets for cancer chemoprevention."
Bode, A. M. and Dong, Z., (2013). Progress in Chemistry 25(9): 1501-1516.

H.I. No. 1952
"Neoalbaconol induces energy depletion and multiple cell death in cancer cells by targeting PDK1-PI3-K/Akt signaling pathway."
Deng Q, Yu X, Xiao L, Hu Z, Luo X, Tao Y, Yang, L., Liu, X., Chen, H., Ding, Z., Feng, T., Tang, Y., Weng, X., Gao, J., Yi, W., Bode, A. M., Dong, Z., Liu, J., Cao, Y. (2013). Cell Death and Disease 4(9): e804.

H.I. No. 1953
"Ribosomal s6 protein kinase 4: A prognostic factor for renal cell carcinoma."
Fan, L., Li, P., Yin, Z., Fu, G., Liao, D. J., Liu, Y., Zhu, J., Zhang, Y., Wang, L., Yan, Q., Guo, Y., Shao, C., Huang, G., Wang, Z. (2013). British Journal of Cancer 109(5): 1137-1146.

H.I. No. 1954
"CDK2 and mTOR are direct molecular targets of isoangustone A in the suppression of human prostate cancer cell growth."
Lee, E., Son, J E, Byun, S., Lee, S J, Kim, Y. A., Liu, K., Kim, J., Lim, S S, Park, J H Y, Dong, Z., Lee, K W, Lee, H J (2013). Toxicology and Applied Pharmacology 272(1): 12-20.

H.I. No. 1955
"Akeratin 15 containing stem cell population from the hair follicle contributes to squamous papilloma development in the mouse."
Shulan, L., Park, H., Trempus, C. S., Gordon, D., Liu, Y., Cotsarelis, G., Morris, R. J. (2013). Molecular Carcinogenesis 52(10): 751-759.

H.I. No. 1956
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Average Impact Factor FY 14

The Hormel Institute Seminars

July 1, 2013 — June 30, 2014

Ningling Kang, Ph.D.
Mayo Clinic
July 23, 2013
"Molecular mechanisms of hepatic stellate cell activation and liver metastic growth"

Chia-Hsin Chan, Ph.D.
University of Texas M.D. Anderson Cancer Center Unit
July 25, 2013
"Skipping cancer: decoding novel regulatory models and targeting SKP2SCF E3 ligase in cancer"

Feng Wang-Johanning, M.D., Ph.D.
University of Texas M.D. Anderson Cancer Center Unit
July 31, 2013
"Novel viral targets for early detection and immunotherapy of cancer"

Andrew Truman, Ph.D.
University of Chicago, IL
August 5, 2013
"Dealing with difficult clients: modulating Hsp70 function as a novel anti-cancer strategy"

Jindan Yu, M.B., Ph.D.
Northwestern University, IL
August 8, 2013
"Genomics and epigenomic regulation of prostate cancer"

Michael Velarde, Ph.D.
Buck Institute for Research on Aging, CA
August 15, 2013
"Cellular senescence beyond tumor suppression: the case of the aging skin"

Natasha Kyprianou, Ph.D.
Medicine and Toxicology
University of Kentucky at Lexington
September 23, 2013
"Impact of taxane chemotherapy on AR - targeting in prostate cancer"

Susan Gilmour, Ph.D.
Lankenau Institute for Medical Research
Jefferson Kimmel Cancer Center
October 24, 2013
"Role of polyamines as strong modifiers of the inflammatory microenvironment of tumors"

Myles Axton, Ph.D.
Editor
November 12, 2013
Nature Genetics

Gary Stoner, Ph.D.
Medical College of Wisconsin
February 14, 2014
"Cancer prevention: dietary factors and pharmacology"

Robert L. Binder, Ph.D.
The Procter & Gamble Company
February 25, 2014
"Transcriptomic profile pattern matching applied to epidermal homeostasis: a powerful approach to identify potential therapeutic agents and for developing mechanistic understanding"

Christina Hoven, Ph.D.
Columbia University, Mailman School of Public Health
June 9, 2014
"Suicide among young people in Sughd Region, Tajikistan"

Peter Vogt, Ph.D.
The Scripps Research Institute, CA
June 10, 2014
"The PI3K pathway as a cancer target"

Income from Grants, Contracts and Development

NATIONAL INSTITUTES OF HEALTH

National Cancer Institute

Anticarcinogenic Mechanisms of Tea Constituents (Z. Dong)	*$2,198*
Chemoprevention of Skin Cancer (Z. Dong)	*$31,071*
*Prevention of PTEN Deletion Driven Prostate Cancer by Selenium (Y. Deng)**	
Prevention of Mammary Tumors by Metformin in Comparison to Calorie Restriction (M. Cleary)	*$176,743*
Gain of Function Mutant p53 Telomere Uncapping-driven Breast Tumorigenesis (Y. Deng)	*$171,366*
Bioactive Compound Modulation of Epigenetic Regulator Sp1/NFkB/miR Network in AML (S. Liu)	*$40,381*
Targeting Aberrant Epigenetics by Nanomedicine (S. Liu)	*$257,921*
Molecular Mechanisms and Targets of Soy Compounds in Colon Cancer (Z. Dong)	*$97,160*
Developing New Ornithine Decarboxylase Inhibitors to Prevent Skin Cancer (Z. Dong)	*$171,913*
Prevention of Prostate Carcinogenesis by Next-generation Selenium (Y. Deng)	*$61,170*
Modulation of p53 Induction by Targeting Cap-dependent Translation in Cancer (D. Yang)	*$31,065*
Hepatic Stellate Cell Regulation of Metastatic Growth in the Liver (N. Kang)	*$129,260*

National Institute of Diabetes and Digestive and Kidney Diseases

Regulation of Tumor Associated Macrophages by A-FABP in Obese Mice (B. Li)	*$21,262*

National Institute of General Medical Sciences

Glycolipid Transfer-Regulation by Membrane Interfaces (R. Brown)	*$240,629*

National Institute of Arthritis and Musculoskeletal and Skin Diseases

Identification of a Keratinocyte Stem Cell Regulatory Gene (R. Morris)	*$287,637*

AgStar Fund for Rural America

AgStar Research Project (A. Bode)	*$16,086*

American Cancer Society

Translational Regulation of p53 Induction in Response to Cellular Stress (D. Yang)	*$24,317*

American Diabetes Association

Structural and Functional Studies of HNF 1alpha and HNF 4alpha (Y. Chi)	*$14,852*

Department of Defense – U.S. Army

RNA Chimeras as a Gene Signature of Breast Cancer (D.J. Liao)	*$81,304*
A Novel Mechanism for the Pathogenesis of Non-melanoma Skin Cancer (R. Morris)	*$84,986*

Mayo Clinic *(S. Liu)* *$7,341*

National Multiple Sclerosis Society

Regulation of T Cell Subset Differentiation by Epidermal Fatty Acid by E-FABP (B. Li)	*$106,122*

Pediatric Pharmaceuticals

*Ginger as an Anti-cancer Agent (A. Bode) **

University of Alabama at Birmingham

Preclinical in vitro and in vivo Agent Development Assays (A. Bode)	*$18,458*

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*(Z. Dong) **

Other Resources

The Hormel Foundation	*$4,041,642*
University of Minnesota	*$440,000*
Indirect Cost Return	*$897,885*
Eagles Cancer Telethon	*$243,000*
Mayo Clinic Collaborative Donation	*$1,000,000*
Other Resources	*$799,568*
Total	**$9,495,337**

** Full award amount stated in previous report*

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