The Hormel Institute

University of Minnesota

IMPROVE THE HEALTH OF THE WORLD

ANNUAL REPORT

09

The stunning completion of The Hormel Institute Expansion Project made for a year of joyful celebration, historic progress and great appreciation for the beautiful, world class facility that was produced.

The $23.4 million project was completed in 2008 and celebrated throughout October with a month of exciting grand opening events. Included in this celebration was another first – a three day International Cancer Research Conference held in Austin, Minnesota, which brought top researchers from around the world to our community.

The addition of new labs allowed for additional research sections to be added. In 2008-2009 The Hormel Institute reached double the number of departments it had in 2006 when the expansion began – each devoted to breaking new ground in cancer research.

The Hormel Institute, designed to support cutting-edge medical research, is the collaborative accomplishment of many. We remain deeply grateful to our leaders, our community, our partners and our collaborators for this gift.

Indeed your support allows today's research to flourish – and provides the path for tomorrow's cures to come.

Our research is dedicated to you.

— The Hormel Institute, University of Minnesota

Message from the Director

Dr. Zigang Dong

The fiscal year of 2008-09 was another wonderfully successful year for The Hormel Institute. We continue to enjoy good fortune, happiness, and growth. Under the leadership of Mr. Richard L. Knowlton, The Hormel Foundation along with Hormel Foods Corporation, the City of Austin, Mower County, and many generous donors from Austin and other parts of the country have provided funding for a new two-story building and complete renovation of the existing buildings. Under the supervision of Mr. Gary Ray, the building was completed in July 2008.

The Hormel Foundation further strengthened the partnership with the University of Minnesota and Mayo Clinic under a new collaborative agreement signed by Mr. Richard L. Knowlton, Chairman of The Hormel Foundation, Dr. Robert Bruininks, President of the University of Minnesota, and Dr. Glenn Forbes, President and CEO of Mayo Clinic. This agreement strengthens and solidifies the collaborations between The Hormel Institute, other University departments, and the Mayo Clinic. Mayo Clinic announced a $5 million donation to The Hormel Institute for support of cancer research and collaborations between the Institute and Mayo Clinic.

Dr. Tim Mulcahy, Vice President for Research of the University of Minnesota and Chairman of The Hormel Institute Board of Directors, continues to strongly support The Hormel Institute with matching funds for numerous pieces of basic and high-end research equipment, including a spectrophotometer–all of which were desperately needed for the continued research success of The Hormel Institute. Most importantly, Dr. Mulcahy procured and allocated $3.85 million in support of the hiring of new faculty members at The Hormel Institute.

The Hormel Institute continues to enjoy its leadership in the scientific field showing that dietary factors modulate crucial cellular signal transduction pathways in cancer development and prevention. The Institute was one of the first to report the discovery of key molecular targets and mechanisms in tumor promotion. The Hormel Institute is rapidly gaining a solid reputation as a leading research institute making major contributions to the identification and characterization of natural chemopreventive agents that are nontoxic but highly effective as anticancer agents. In May 2009, the Molecular and Cellular Biology section published work that was featured on the cover of Cancer Research.

By focusing on cancer, The Hormel Institute has experienced a dramatic increase in external research funding even in the national environment of overall decreased funding for research. Currently, all sections have grants from NIH and/or other funding agencies.

The official dedication and grand opening of The Hormel Institute Expansion Project was celebrated October 3, 2008 with 400 supporters in attendance. Leaders throughout the state were present for The Hormel Institute Dedication, including The Hormel Foundation Chair Richard L. Knowlton, Hormel Foods Corporation Chairman of the Board, President and CEO Jeffrey L. Ettinger, Governor Tim Pawlenty, Senator Amy Klobuchar, University of Minnesota President Robert Bruininks, Mayo Clinic Chief Executive Officer Dr. Glenn Forbes and Hormel Foods' heir James Hormel.

"The effects of The Hormel Institute Expansion are multi-dimensional," said Richard L. Knowlton, Chairman of The Hormel Foundation and project leader. "It has strengthened partnerships between The Hormel Institute, the University of Minnesota and Mayo Clinic (known as the Bioscience Triad), improved science education within the Austin school system and enhanced the community and region. We believe this expansion is a starting point for continued growth and achievement for The Hormel Institute."

"Hormel Foods and The Hormel Institute share a great history, and we are excited to be part of this momentous occasion," Ettinger said. "Paraphrasing the words spoken by H.H. Corey, former CEO of Hormel Foods at the dedication in 1949, we believe this expansion will continue to benefit all of mankind. We wait with anticipation along with the rest of the world to see what the group will discover."

Dr. Forbes characterized The Hormel Institute collaboration as in keeping with the Mayo Clinic tradition of collaboration and the reason for the financial support. "The Hormel Institute expansion is a welcome development, not only for Dr. Dong and his staff, but for all of their collaborators. Mayo is proud to be one of those collaborators and we look forward to continuing our relationship of scientific achievement."

The University of Minnesota regards The Hormel Institute as one of the research units that will help the University reach its goal of becoming one of the top three public research universities in the world within 10 years. "The expansion of The Hormel Institute builds on the momentum of the past decade at the University of Minnesota," said Robert Bruininks, President of the University of Minnesota. "Our state has the second most vibrant biomedical sciences corridor in the country, and the groundbreaking research conducted at The Hormel Institute and in our laboratories statewide will continue to make vital contributions to the treatment and prevention of cancer and other chronic diseases, as well as Minnesota's economy and quality of life."

In conjunction with the grand opening, on October 4-6, 2008 The Hormel Institute hosted the joint 3rd Hormel Institute Frontiers in Cancer Research and 8th International Skin Carcinogenesis Conferences in Austin, MN. More than 200 scientists from different countries attended the conferences and discussed their findings in the identification of promising molecular targets and the development of agents with the goal of effectively transitioning these into the clinical setting. Drs. Bode and Dong summarized the discussion of the conference and published a report entitled, "Signal transduction molecules as targets for cancer prevention" in Science Signaling.

The Hormel Institute and Mayo Clinic have established an Office of Translational Research onsite at The Hormel Institute. The purpose of this office will be to facilitate movement of basic research findings into the clinic. Other important collaborations include an effort between The Hormel Institute, the U of M Supercomputing Institute, and IBM to identify and delineate key protein-protein interactions and protein-small molecule interactions by computer simulation and then to validate the interaction in vivo. In collaboration with IBM, The Hormel Institute became the first research facility (January 2008) in the state of Minnesota to own the world's most powerful supercomputer: the IBM BlueGene/L. The purchase of the BlueGene/L is the first step in establishing an International Center for Research Technology (ICRT) to be housed at The Hormel Institute in Austin, Minnesota in collaboration with The Development Corporation of Austin (DCA), MN. The ICRT will provide the most cutting edge tools of technology available today to researchers working at biobusinesses, medical centers, colleges and universities in the southern region and statewide. In particular, the ICRT will work with manufacturers of technology, like IBM Rochester, to develop new technologies to accelerate discovery and facilitate comprehensive study of human disease by combining analyses of protein structure/function with advanced methods of data management and drug screening. The ICRT will also work with smaller biobusinesses in the state and region to provide consultation and services. The net result will be a greater understanding of biological systems for improving the quality of life in Minnesota, the nation, and the world and a dramatic, positive impact on economic development in Bioscience and Biotechnology for the state of Minnesota. During this last fiscal year, U.S. Congressman Tim Walz visited The Hormel Institute twice and showed very strong support to the Institute by acquiring funding of over $700,000 toward the purchase of high-end instrumentation in establishing the ICRT.

All of these accomplishments would not be possible without the generous ongoing support of The Hormel Foundation and Hormel Foods. In particular, I would like to thank Mr. Richard Knowlton for his continued interest and support of the Institute, Mr. Joel Johnson, Mr. Jeff Ettinger, Mr. Gary Ray and Mr. Larry Pfeil for their generous support, and Dr. Tim Mulcahy for his leadership and support. We thank our elected leaders, U.S. Representative Tim Walz, U.S. Senator Amy Klobuchar, Minnesota State Senator Dan Sparks, and Minnesota State Representative Jeanne Poppe. The Hormel Institute is a team project. By working together, we will help to lead our university in realizing the goal of becoming a top research institute worldwide.

CELLULAR AND MOLECULAR BIOLOGY

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

Cancer is one of the leading causes of death in today's world. This deadly disease can happen in men and women, black and white, rich and poor, people in developed and developing countries. Almost every one of us has had the experience of losing a friend or family member to cancer. In order to facilitate the development of chemopreventive and chemotherapeutic agents that specifically target molecules important in cancer development, we must know the enemy—we must understand carcinogenesis. The prevailing thought today is that cancer can be prevented or treated by targeting specific cancer genes, signaling proteins and transcription factors.

By focusing on the molecular mechanisms explaining how normal cells can undergo neoplastic transformation induced by tumor promoters, we have discovered that several specific transcription factors and protein kinases are critical factors in cancer development and significant targets for cancer prevention and treatment.

Skin cancer is the most common human malignancy in the world and is the number one cancer in the terms of incidence in the USA. The major etiological factor of human skin cancer is the chronic exposure to UV light from sun. UV irradiation is categorized by wavelength as UVA I (340-400 nm), UVA II (320-340 nm), UVB (280-320) nm), and UVC (180-280 nm). In mouse skin, UV light acts as both an initiator, presumably by causing DNA damage leading to gene mutations, and as a tumor promoter. The mechanisms behind the tumor promoting ability of UV are areas of intense study in our laboratory. Numerous oncogenic and/or protective signaling pathways are activated in UV-induced carcinogenesis. Very little is known about UV-induced phosphorylation of histones, proteins that are very important in the packaging of DNA.

We found that the UV-induced signal transduction pathways are mediated primarily through signaling cascades involving the mitogen-activated protein (MAP) kinases, resulting in the modification of transcription factors, including activator protein-1 (AP-1), nuclear factor-kappaB (NF-κB), signal transducer and activator of transcription (STATs), p53 and nuclear factor of activated T cells (NFAT). We also found that histone phosphorylation is critical to mediate UV or other tumor promotion induced apoptosis and cancer formation.

We found that cannabinoid receptors 1/2 (CB1/2), epidermal growth factor receptor (EGFR) and protein kinase C can be directly activated by UV irradiation. The absence of cannabinoid receptors 1 and 2 (CB1/2) in mice results in a dramatic resistance to UV-induced inflammation and marked decrease in UV-induced skin cancer.

The transient receptor potential channel vanilloid subfamily 1 (TRPV1) belongs to a diverse family of proteins comprised of non-selective cation channels. The TRPV1 is not only expressed in neuronal tissues, but has also been detected in epidermis, dermal blood vessels, normal human keratinocytes, mast cells, appendage epithelial structures, human cultured fibroblasts, human hair follicles, human lung BEAS-2B cells, and HaCaT cells, but the function of TRPV1 in non-neuronal cells and tissues is unclear. Notably, the absence of TRPV1 in mice results in a striking increase in skin carcinogenesis.

Left to right front row: Wei-Ya Ma, Yanming Xu, Joon Twayana (Intern)

Left bench: Naomi Oi, Kun Yeong Lee, Margarita Malakhova, Myoung Ok Kim, Andria Carper

Right bench: Jixia Li, Molly Pogones (Intern), Leah Walters (Intern), Dasha Urusova, Priya Rathinaswamy

Second from last row: Jong Eun Kim, Sung-Hyun Kim, Sung Young Lee, Nu Ry Song, Shengqing Li, Ke Yao, Hong-Gyum Kim, Charles Lee (Intern), Zigang Dong, Ann Bode, Tao Yin (Andy) Lau, Tatyana Zykova, Sung Keun Jung, Chul Ho Jeong, Angelo Pugliese

Last row: Ji-Shuai Zhang, Cong Peng, Kang Dong Liu, Sanguine (Sam) Byun, Young Jin Jeon, Paskal Pandey (Intern), Dong-Joon Kim, Madhu Mottamal, K. Srinivasa Reddy

Not pictured: Yong Yeon Cho

Because of their analogous expression in apparently almost every tissue, JNK1 and JNK2 have most often been considered to have overlapping or redundant functions. However, JNK1 deficient mice develop more UVA-induced papillomas than either JNK wildtype or JNK2 deficient mice supporting a specific role for JNK1 and JNK2 in tumorigenesis.

Another major goal of our section is to identify anticancer agents that have low toxicity with fewer adverse side effects, which may be used alone or in combination with traditional chemotherapeutic agents to prevent or treat cancer. Many dietary factors have potent anticancer activities that work through, as yet, unknown mechanisms. Various dietary factors, including many isolated from green and black tea, potatoes, broccoli, peanuts, ginger root and rice, can have effects on key signaling molecules crucial in cancer development and prevention.

Over the last few years, our laboratory has shown that various specific kinases and their target substrates appear to exhibit a distinctive activity or higher expression in cancer tissues compared to normal tissues; and therefore might be excellent targets for chemopreventive agents. These proteins include vimentin,

glucose-regulated protein 78 (GRP78), insulin-like growth factor receptor 1 (IGF-1R), paxillin, T-LAK cell-originated protein kinase (TOPK), c-Jun N-terminal Kinase 2, the zeta chain associated protein of 70 kDa (ZAP-70), cyclin-dependent kinase 3(Cdk3), CB1/2, C-terminal Src kinase (CSK), and the transient receptor potential channel vanilloid subfamily 1 (TRPV1).

Vimentin, one of the type III interfilament proteins, is a major component of intermediate filaments (Ifs), and is expressed during development in a wide range of cells, including mesenchymal cells and in a variety of cultured cell lines and tumors. Accumulation of vimentin is frequently noted as a pathological hallmark in a wide range of human diseases.

GRP78 is aberrantly induced in cancer and has been shown to be correlated with resistance to chemotherapeutic agents, including doxorubicin, etoposide and adriamydin. The induction of GRP78 is associated with enhanced cancer cell survival.

The IGF-1R is a tyrosine kinase receptor that is activated by the binding of secreted growth factors, IGF-1 or IGF-2. Impairment of IGF-1R signaling in various cancer cell lines caused inhibition of the transformed phenotype as determined by the inhibition of colony formation in soft agar and the inhibition of tumor formation in athymic nude mice.

Paxillin is a substrate for the oncogenic tyrosine kinase v-Src in v-Src transformed cells and acts as a transducer for activation signals from receptor tyrosine kinases to downstream MAP kinases such as the c-Jun N-terminal kinases, suggesting that paxillin is involved in cell transformation and tumor development.

TOPK is overexpressed in highly proliferating tumors such as leukemias and myelomas, and appears to play a key role in tumorigenesis or metastasis. Cell lines expressing high levels of TOPK are more resistant to arsenite-induced apoptosis than cell lines with low TOPK expression. TOPK is also highly expressed in human colorectal cancer tissues and cell lines and plays an important role in the transformation of colorectal cancer. TOPK promotes transformation in vitro and in vivo and knockdown of TOPK in HCT116 colorectal cancer cells reduces this cell line's tumorigenic properties in vitro and in vivo.

ZAP-70 is a Syk (spleen tyrosine kinase) family typosine kinase, which is associated with the ζ subunit of the T cell receptor (TCR). The ZAP-70 protein is primarily expressed in T and natural killer (NK) cells and plays an essential role in signaling through the T cell antigen receptor. Notable, ZAP-70 is highly expressing in leukemias.

Cdk3, a member of the cdk family of kinases, plays a critical role in cell cycle regulation and is involved in G0-G1 and G1-S cell cycle transitions. Cdk3 is over-expressed in glioblastoma tissue and various cancer cell lines.

CSK can bind with c-Jun and phosphorylate c-Jun at Y26 and Y170. Phosphorylation of c-Jun by CSK, opposite to JNK1 and ERKs, promoted its degradation and reduces c-Jun stability. By promoting c-Jun degradation, CSK helps to maintain a low steady-state level of c-Jun and inhibits AP-1 activity and cell transformation caused by c-Jun, and thus controls cell proliferation under normal growth conditions. A loss of functional CSK is related to increase carcinogenesis.

We have also focused on the effects of tea in inhibiting carcinogenesis. We have reported that (-)-epigallocatechin-3-gallate (EGCG) from green tea or theaflavins (TFs) from black tea inhibit tumor promoter induced AP-1, NFκB activation, MAP kinase activation and cell transformation. Searching for the EGCG "receptor" or high affinity proteins that bind to EGCG is the first step in understanding the molecular and biochemical mechanism of the anticancer effects of tea polyphenols. Recently, we identified the intermediate filament protein, vimentin, GRP78, ZAP-70, and insulin like growth factor receptor 1 (IGF-1R), as novel EGCG-binding proteins. Intermediate filament (IF) proteins, such as vimentin, have an important functional involvement in cell division and proliferation. EGCG has been reported to inhibit cell proliferation of a variety of cell lines and in our work, when vimentin expression was suppressed, cell growth was inhibited.

More recently, we have worked with Dr. Yuan-Ping Pang at Mayo Clinic to use high-performance computers, modern chemical synthesis and cancer biology to block JNKs and develop anticancer drugs. Further, we have teamed with IBM and its Blue Gene group under the leadership of Mike Good and the University of Minnesota Super Computing Institute to use the world's fastest computer to understand complex diseases like cancer; and to screen anticancer drugs for cancer prevention and treatment. In collaboration with Dr. Paul Limburg (Mayo Clinic Rochester), we will conduct clinical trials to use cancer preventive agents developed in our Institute.

In summary, we address fundamental questions concerning the response of animal and/or human cells to carcinogens and tumor promoters such as UV light, arsenic, TPA and growth factors. We have established a series of necessary models and systems, such as the over-agar assay for cell transformation, gene knockout mice, transcription factor/luciferase promoter stably transfected cells and transgenic mice, as well as gene knockdown (siRNA) or dominant negative mutant stably transfected cell lines. These models have been extensively utilized to examine the tumor promoter-induced signal transduction pathways and their role in cell neoplastic transformation. We have systematically studied the signal transduction networks induced by UVA, UVB and UVC. Such studies have provided the basis for the carcinogenic process caused by environmental carcinogens and molecular mechanisms for cancer prevention.

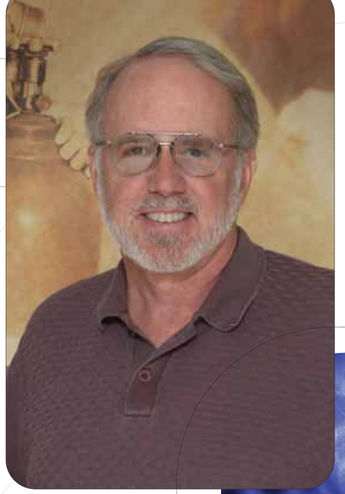
Nutritional or dietary factors have attracted a great deal of interest because of their perceived ability to act as highly effective chemopreventive agents. They are perceived as being generally safe and may have efficacy as chemopreventive agents by preventing or reversing premalignant lesions and/or reducing second primary tumor incidence. Many of these compounds appear to act on multiple tumor promoter-stimulated cellular pathways. Some of the most interesting and well-documented are resveratrol and components of tea, EGCG, theaflavins and caffeine. Other potentially effective dietary compounds include inositol hexaphosphate, ginger and CAPE. A continuing emphasis on obtaining rigorous research data and critical analysis of those data regarding these and other food factors is vital to determine the molecular basis and long-term effectiveness and safety of these compounds as chemopreventive agents. Large-scale animal and molecular biology studies are needed to address the bioavailability, toxicity, molecular target, signal transduction pathways, and side effects of dietary factors. Clinical trials based on clear mechanistic studies are also needed to assess the effectiveness of these dietary factors in the human population. By doing so, we will develop more potential cancer preventing agents with few side effects.

Other Professional Activities
Zigang Dong

Grant Reviewer
- National Institutes of Health

Editor, Associate Editor or Editing Board Member
- Cancer Research
- Cancer Prevention Research
- Molecular Carcinogenesis
- Carcinogenesis
- International Journal of Biological Sciences
- Food Factors

BIOPHYSICS
Section Leader
Howard L. Brockman, Ph.D.
Professor

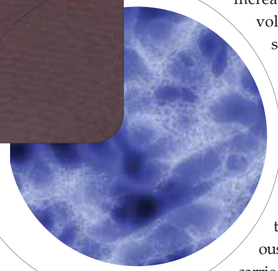


Our lab's main research interest is elucidation of interfacial structure and regulation of enzyme catalyzed reactions occurring at interfaces: investigation of the interactions of soluble proteins with interfaces, the relation of interfacial structure to catalytic properties of enzymes at interfaces and lipid-mediated signal transduction. We also design instrumentation and methods for surface chemical analysis.

The key component at the heart of the novel instrument on which our work depends is a flow cell that maintains a constant depth (~150 um) of the liquid flowing through it and is open on the upper surface to the atmosphere. The interface between the flowing liquid and the air serves as a renewable platform at which lipids or proteins can be immobilized in the interface by self or directed assembly. Relevant examples of such assembly are the formation of an antibody monolayer by adsorption from the aqueous phase and the formation of a lipid monolayer by spreading of lipid onto the surface from a volatile organic solvent. Subsequently, the adsorption of solute, e.g. a protein, to this interface from the aqueous phase can be optically measured.

Last year we described improvements in this flow cell and associated instrumentation that increased sensitivity and stability. We showed further that it can be used to study antigen-antibody interaction at picomolar concentrations as a model binding reaction for analyzing instrument performance. Further study of this interaction revealed more subtle and previously unrecognized environmentally-dependent drifts and perturbations. By improvement of temperature control and redesigning the electronic circuit that controls liquid level, these have now been eliminated. Constant liquid level and constant fluorescence background, essential to the use of the instrument, can be maintained for days.

Study of the antigen-antibody model reaction over long time periods also revealed a potential limit of the flow cell. Specifically, not only did antigen-antibody binding occur at the air-water interface but, with time, it also bound to the glass bottom of the trough. Covalently modifying the glass with polyethylene glycol chains eliminated much of this undesirable binding. However, the modified glass surface deteriorated over time, particularly with the use of harsh cleaners during the regeneration cycle. To address this we developed a novel flow cell in which the depth of part of the cell is increased and that resulting new volume is filled by flowing 2M sucrose. Effectively, the solute-bearing upper solution flows over the denser sucrose that is moving in the same direction at approximately the same speed. If any solute adsorption to the sucrose-buffer interface were to occur, it would be continuously and rapidly (~10 mm/sec) carried away from the 1 mm diameter site, just after the flows converge, at which the surface is probed optically. Experimental data now shows that the accumulation of solute we measure optically is occurring only at the gas-liquid interface.



In other work we have collaborated with others to study the physical role of oxysterols in regulating cellular cholesterol homeostasis. Our role in this collaboration was to show that 25-hydroxycholesterol and its enantiomer interact identically with cholesterol and membrane relevant phospholipids. That the biological response of cells to both agents is identical supports the idea that a protion of the regulation of cholesterol homeostasis by oxysterols does not involve stereospecific interaction of the oxysterol with a protein component.

Left to right: Dmitry Malakhov, Maureen Momsen, Howard Brockman, Nancy Mizuno, Bill Momsen

Other Professional Activities
Howard Brockman

Member:
- NIH Special Emphasis Panel/Scientific Review Group, October 9-10, 2008.

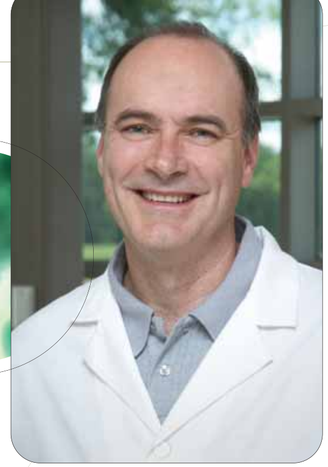
Invited Speaker:
- ACS Colloid and Surface Science Symposium June 15-18, 2008, Raleigh, NC. "A Microfluidic Platform for Characterizing Protein and Surfactant Adsorption to Gas-Liquid Interfaces"

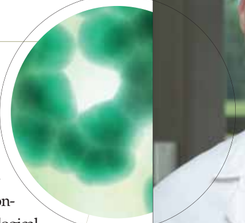
MEMBRANE BIOCHEMISTRY

Section Leader

Rhoderick E. Brown, Ph.D.

Professor





Our lab research interests include structure–function relationships of sphingolipid binding/transfer proteins; protein-mediated and spontaneous intermembrane lipid transfer with emphasis on glycolipids and gangliosides; intracellular lipid transport; structure-function relationships of glycolipids and sphingolipids in biological and model membranes as related to sphingolipid-sterol membrane domains, i.e. 'rafts'; nanotechnology and nanofabrication processes involving glycolipids.

Our progress over the past 4-5 years is directly responsible for human glycolipid transfer protein (GLTP) becoming recognized as the prototype and founding member of the new GLTP superfamily of proteins, based on its unique conformational fold among lipid binding/transfer proteins and characterized by a novel membrane interaction site. The GLTP-fold occurs widely among eukaryotes and provides key functionality to larger proteins (e.g. FAPP2) that play key roles in glycosphingolipid synthesis.

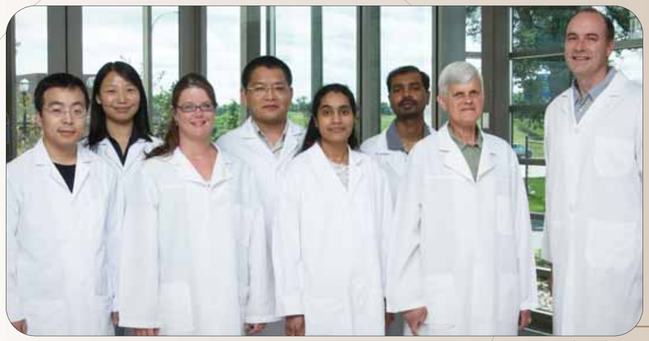
Glycolipid Transfer Protein (GLTP) and Related Homologs

GLTP is a small (~24 kDa) and soluble protein that selectively accelerates the transfer of glycolipids between membranes. GLTP specificity for glycolipids is broad, encompassing both sphingoid-based and glycerol-based glycolipids with β-linked sugars. This specificity includes neutral and anionic glycolipids such as gangliosides, but not sphingomyelin, phosphoglycerides or neutral lipids. The novel conformational fold used by GLTP to ligand glycolipid was first reported in our 2004 Nature paper, confirming that GLTP differs distinctly from other soluble glycolipid-binding proteins such as nonspecific lipid transfer protein, lysosomal sphingolipid activator proteins, lectins, and lung surfactant proteins as well as from other lipid binding/transfer proteins. Studies currently underway are aimed at elaborating the mechanism by used by GLTP to acquire and release glycolipids and its physiological importance in normal and diseased cells.

Human GLTP Mechanism of Action

Our past studies of glycolipid uptake rates by GLTP and assessment of GLTP binding affinity for various glycolipids involved characterization of signature changes in intrinsic Trp fluorescence triggered by glycolipid liganding. From our X-ray crystallographic studies of human GLTP, we knew that Trp96 and Trp142 are located near each other (14-16Å) within a surface region that forms a putative membrane interaction site. Trp 96 is also a key residue of the glycolipid liganding site, serving as a stacking platform for the initial ceramide-linked sugar and facilitating formation of multiple hydrogen bonds between the sugar ring hydroxyls and Asp48, Asn52, and Lys55. The third Trp residue (Trp85) projects towards the protein interior and stacks against Pro86 (cis configuration), limiting access to the protein surface and strongly quenching it. The Trp emission peak of wtGLTP was known to undergo a substantial blue shift in wavelength maximum (λmax) and decrease in intensity (~40%) when the glycolipid-free form of wtGLTP interacts with membranes containing glycolipid. What remained unclear is whether the changes in Trp fluorescence reflect interaction with the membrane or glycolipid uptake by GLTP. We showed that Trp96 dominates the average Trp emission signal of wtGLTP and that the glycolipid binding event is chiefly responsible for the observed changes in Trp fluorescence, enabling assessment of GSL liganding affinity as well as comparison of the impact of changing glycolipid structure (e.g. either sugar headgroup or acyl chain composition) on binding affinity to GLTP. The strong selectivity for glycolipids makes GLTP a potentially useful biotechnical tool for molecular manipulation of GSL-enriched microdomains in membranes.

We also investigated whether GLTP can form dimers in solution. FPLC size exclusion chromatography of GLTP (at high protein conc.) yielded peaks corresponding in size to monomer, multimer, and intermediate mass. However, evaluation by ESI-MS (via a new collaboration with the Mayo Proteomics Research Lab; Dr. Bob Bergen) revealed no GLTP dimer. Also, native gel electrophoresis showed that the intermediate protein peak migrated as monomer, ruling out GLTP dimerization and raising the possibility of a molten globule-like conformation. We investigated further. Intrinsic GLTP Trp fluorescence showed a blue-shifted (~2nm) emission λmax, indicating an altered Trp environment. ANS binding resulted in a large blue shift (~20nm) in λmax and dramatically enhanced emission intensity (~120%). Far-UV-CD showed retention of ordered secondary structure (>95%), but substantially reduced cooperativity during thermally-induced unfolding. Unfortunately, the induced optical activity of GLTP Trp/Tyr signal (near-UV-CD) was insufficient to reliably assess changes in tertiary structure. To further characteize GLTP unfolding intermediates, the effect of urea was studied. Trp emission changes suggested a two-step unfolding pathway involving intermediate formation at 4M urea and characterized by blue-shifted Trp emission. Far-UV-CD analyses of the 4M urea-induced intermediate indicated diminished secondary structure and cooperative melting at lower temperature compared to native GLTP, but the near-UV-CD signal did not provide definitive insights into tertiary folding status. ANS binding showed a λmax blue-shift (1nm) and 60% increase in emission intensity compared to untreated GLTP. The data are consistent with GLTP transitioning to molten globule-like and other partially unfolded states. Studies on the significance of these intermediates with respect to function are underway. This work represents the first insights into GLTP stability and folding/unfolding.



Left to right: Yong-Guang Gao, Xiuhong Zhai, Tawnya Gardner (Intern), Xianqiong Zou, Roopa Kenoth, Ravikanth Kamlekar, Helen Pike, Rick Brown

Mapping the membrane interaction region of human GLTP by site-directed mutagenesis

The surface region surrounding the glycolipid liganding site has many nonpolar amino acids along with multiple tyrosines, tryptophans and lysines, typical residues of membrane interaction motifs. The GLTP conformational fold that spatially organizes these residues clearly differs from other membrane targeting motifs, such as the protein kinase C homology -1 and -2 (C1, C2) domains of many phospholipases and protein kinases, as well as the FYVE, pleckstrin homology (PH), and phox (PX) domains.

Our past studies identifying potential surface regions of GLTP likely to be involved in membrane interaction and/or dimerization involved modeling approaches using the Optimal Docking Area algorithm and the Orientations of Proteins in Membranes (OPM) computational approach for analyzing the spatial arrangement of proteins in lipid bilayers. The modeling data support a membrane 'docking' role for Trp142 (and possibly Trp96) in GLTP during glycolipid acquisition. To directly assess the roles of Trp142 and Trp96 in membrane interaction, point mutational studies have been ongoing as well as measurements of glycolipid transfer activity, intrinsic fluorescence and CD spectroscopy. Compared to wtGLTP, the single Trp mutant, W96Y, retains 65% activity; whereas the double Trp mutants, W85Y-W96F & W96F-W142Y, retain 22% and 110% activities. Quenching with acrylamide or potassium iodide at physiological ionic strength results in linear Stern-Volmer plots, indicating accessibility of emitting Trp residues to soluble quenchers and consistent with native-like folding. CD data reveals significant differences in W85Y-W96F-GLTP secondary structure compared to wtGLTP; whereas W96F-W142Y-GLTP and W96Y-GLTP retain native secondary structure. The negative outcome of conservative mutation of Trp 85 suggests a crucial role in proper folding of GLTP; whereas, the tolerance of Trp96 and Trp142 for conservative, but not radical mutation (e.g. change to A), is consistent with specialized roles in GLTP function, i.e. glycolipid liganding and membrane interaction.

Fungal and plant GLTP orthologs (HET-C2 & ACD11)

Our ongoing collaboration with the John Mundy lab (Univ. Copenhagen) has resulted in continued progress with ACD11, a plant GLTPortholog. Using GLTP

as a structural template, Nikolaj Petersen developed ACD11 point mutants (R103W, D60V, and H143L) and then cloned, expressed, purified, and analyzed their transfer activities. Previously, we had shown that wtACD11 does not transfer glycolipids or ceramide but accelerates sphingosine intervesicular transfer. Since then, we have found that ACD11 can accelerate the intervesicular transfer of sphingomyelin (SM). Using SM as transfer 'substrate', the point mutants of ACD11 have all been analyzed. Only the R103W appears capable of maintaining SM transfer activity. We also determined that human wtGLTP could complement for ACD11 function by reversing the effect of acd11 gene disruption, which is known to promote apoptosis.

Progress has continued with HET-C2, a fungal GLTP-ortholog, previously shown by us to transfer galactosylceramide in vitro. The crystal structure of HET-C2 is unknown, but molecular modeling suggests conservation of the GLTP-fold. The location and functionality of the Trp residues in HETC2 have been investigated. Sequence homology shows one (Trp109) of HET-C2's two Trp residues aligns with GLTP Trp96, which resides in the sugar headgroup binding site. The other HET-C2 Trp does not align with either of GLTP's other two Trps. The Trp fluorescence spectrum of native HET-C2 exhibits a λmax of ~355nm, indicating Trp localization to a polar environment. Acrylamide and KI quenches >90% of the average Trp fluorescence consistent with the HET-C2 Trps being surface localized rather than buried. The linearity of Stern-Volmer plots for native Het- C2 and urea-denatured (8M) HET-C2 suggests collisional quenching at physiological pH and ionic strength. Stern-Volmer constants are higher for native protein than denatured protein. Upon interaction with curvature-stressed POPC vesicles, the Trp λmax of HET-C2 blue-shifts by ~2nm and decreases in intensity (~13.5%). Including glycolipid in the vesicles enhances the blue shift (~3nm) and significantly decreases emission intensity (~21%). Far-UV-CD of HET-C2 shows secondary structure dominated by alpha-helices and a highly cooperative, thermal unfolding transition of ~43°C. HET-C2 secondary structure is unaffected by interaction with vesicles lacking or containing glycolipid. However, the HET-C2 near-UV-CD signal which reflects induced optical activity of Tyr/Trp residues, shows that interaction with vesicles lacking glycolipid results in significantly altered signal that is not observed when the vesicles contain glycolipid. Collectively, the CD data show that phospholipid membranes destabilize of HET-C2 tertiary structure without producing alterations in secondary structure. The membrane interfacial environment seems to trigger molten globule-like conformational changes in HET-C2 that facilitate access to its glycolipid binding pocket. The presence of glycolipid in the vesicles counteracts the destabilizing effect, reflecting stabilization gained by uptake of glycolipid into the binding pocket. Collectively, these findings enable our proposed model for GLTP-fold action to be elaborated: Partitioning of GLTP to the membrane is relatively weak, transient, and nonperturbing, is influenced by membrane lipid composition, and involves a membrane interaction site containing tryptophan(s). Upon translocation to the membrane interface, GLTP transitions to a molten globulelike state and scans the surface until finding and recognizing the carbohydrate moiety on GSL. Formation of a GLTP/glycolipid complex returns the conformation to its pre-loaded state before release into the bulk medium to accomplish GSL transfer.

GLTP Genomics and GLTP Functional Role in Cells

Our past research resulted in the first characterization of human GLTP gene (chromosome 12) and a GLTP pseudogene (chromosome 11). Analyses of gene product in vitro activity, in vivo transcription, and 5'UTR gene methylation status led to clear support for activity by the GLTP12 gene and transcriptional silencing of the GLTP11 pseudogene. Phylogenetic and evolutionary analyses revealed that the single exon GLTP11 pseudogene (GLTPP1) is primate-specific; while the 5-exon/4-intron organization of the actively transcribed GLTP12 gene is widely conserved among vertebrates. Recently, we shifted focus to cloning and characterizing the highly GC-enriched, GLTP gene promoter region to gain understanding about the regulation of GLTP transcriptional activity in vivo. Luciferase reporter constructs show that a 1169bp promoter sequence (-1150 to +19 relative to major transcriptional start site) displays constitutive GLTP promoter activity. A series of 5' deletion and 3' deletion mutants of the 5' flanking region, cloned into pGL3-Basic luciferase plasmids, enabled identification of a 225bp region (from -350 to -126) as the GLTP basal promoter by luciferase reporter assays. A negative regulatory region that downregulates

GLTP promoter activity was found upstream of -416. Using electrophoresis mobility shift assays and chromatin immunoprecipitation assays, multiple GC-box sites specific for Sp1 and Sp3 binding were shown. Mutational analysis confirmed that GLTP basal promoter activity depends on the Sp1 and Sp3 binding sites being functional and showed that mutation of one particular GC-box site was especially detrimental to promoter activity. Overexpression of Sp1 and Sp3 transcription factors also reduced GLTP promoter activity. Collectively, the results provide the first characterization of the human GLTP promoter and identify Sp1 and Sp3 as important transcriptional regulators of the human GLTP gene.

To gain insights into cellular phenotypic changes induced by GLTP expression, we have recently shown that GLTP overexpression in HeLa cells markedly affects cell shape. More than half of transfected cells overexpressing wild-type GLTP show a rounded phenotype within 48 h. However, HeLa cell shape was not altered by overexpression of W96A GLTP, a liganding-site mutation that almost completely blocks glycolipid transfer between membranes in vitro. We also found that the C-terminus of delta-catenin not only interacts with GLTP, but also accelerated the GLTP-induced change in cellular shape. Continuing studies are focused on elucidating the mechanism of the GLTP-induced changes in cell shape. A possible role of GLTP overexpression triggering programmed cell death is now being explored.

Sphingolipid Biophysical Studies

Sphingolipids have been center stage in the lab for the past twenty years. Over the past decade and a half, key roles emerged regarding sphingolipid involvement in the formation of membrane microdomains commonly referred to as rafts. The significance of rafts to cell biology at large lies in their proposed function as a lateral localizing regions for various lipid-anchored signaling proteins. Rafts are of biomedical interest because they may be target sites for various pathogens including certain viruses, bacteria, and prions. The physical environment in raft microdomains is usually depicted qualitatively, i.e. 'detergent-resistant'; 'liquid-ordered', in ways that do not adequately explain their lateral sorting functionality. To address the issue, our strategy has been to introduce well-defined structural variation into sphingolipids by lipid synthetic approaches. Then, we directly assess the impact on intermolecular lateral elasticity within model membrane systems, i.e. monolayers. We have shown that the surface compressional modulus, which reflects lipid lateral packing elasticity, provides a sensitive, reproducible, and quantitative way to define the physical packing environment over a wide range of lipid compositions, including those most relevant to biomembranes. Such insights are not provided by classic monolayer 'area condensation' measurements. Nonetheless, the surface compressional modulus is a macroscopic property that becomes increasingly difficult to interpret at the molecular level as the lipid mixture increases in complexity. To overcome this limitation, we are now developing ways to acquire fluorescence emission spectra of lipids labeled with BODIPYfluorophores directly from lipid monolayers to better understand how changes in lipid composition known to affect the surface compressional moduli impact lipid local concentrations. The development and use of tetramethyl-BODIPY lipid derivatives (linked to PC, sphingomyelin, and GalCer) provides new fluorescent probes that efficiently embed into the lipid hydrocarbon region and show no tendency to 'loop back' to the membrane interface. The ongoing work, enabling nano-dimensional localization into the lateral organization of lipid probes in complex lipid mixture, involves collaborations with J. G. Molotkovsky (Russian Academy of Sciences), Howard Brockman (UMNHormel Institute) and Istvan Sùgar (Mt. Sinai Med. Ctr.).

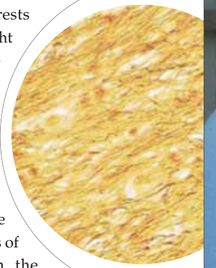
Our expertise in assessing lipid lateral interactions within model membranes has led to a productive collaboration with investigators (Dan Ory, Doug Covey, Paul Schlessinger, and Jean Schaffer) at Washington Univ. The work has involved assessment of lateral interactions of different oxysterols with phosphoglycerides containing varying levels of acyl unsaturation. The work appeared in J. Biol. Chem. early in 2009.

NUTRITION AND METABOLISM

Section Leader

Margot P. Cleary, Ph.D.

Professor



This section's primary interests include the effects of body weight on the development of breast cancer using mouse models. Studies include 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. We are also determining if characteristics of the cancer cells interact with the response to nutrition interventions. These studies are complemented by in vitro tissue culture experiments. Additional investigations are focused on the effect of caloric restriction on the development of breast/mammary tumors and prostate cancer using mouse models. Mechanisms of the protective effect of caloric restriction on cancer development include studies of leptin/leptin receptors, adiponectin/adiponectin receptors and the IGF-axis. Recent studies have expanded to the effects of obesity on prostate cancer development. We are also investigating the effects of specific long chain fatty acids as chemopreventive agents and the effect of reactive oxygen species in the protective effect of calorie restriction.

The major focus of research in the Nutrition and Metabolism Section has been the interaction of caloric intake, changes in body weight and the development of breast cancer. Recently our intervention strategies have been expanded to prostate cancer. Several different approaches are used to assess the effects of caloric restriction, as well as body weight changes and/or weight gain/loss on tumorigenesis. The focus was initially on serum leptin and IGF-I as mediators of tumor growth with recent expansion to an additional protein, adiponectin. Similar to leptin, adiponectin is synthesized in adipose tissue, however, in contrast to leptin, its synthesis declines with increasing body weight and body fat. Furthermore, recent studies indicate that lower serum adiponectin levels are associated with the development of several malignancies, including breast and prostate cancers. Additionally, in vitro studies show that addition of adiponectin reduces cell proliferation of both breast and prostate cancer cells and may enhance cell death.

For our mammary tumor studies, both transgenic mice and xenograft mice models are used. We are currently investigating the fact that intermittent caloric restriction was more protective in the prevention of mammary tumor development than was the same degree of caloric intake imposed by chronic (evenly spaced) restriction. We have completed several longitudinal studies where serum samples were obtained prior to tumor detection in order to identify biomarkers. Some of the results support a role for elevated serum IGF-I levels in the development of mammary tumors as well as alterations in the adiponectin to leptin ratio. In addition a cross-sectional study has been done where blood and tissue samples were obtained over the course of mammary tumor development. We are now analyzing results of a study on the effects of high fat diet feeding during refeeding to determine how this affects mammary tumor development and/or prevention. Additionally, Dr. Michael Grossmann received funding in the past year from the Komen Foundation and is studying the interaction of an omega-3 fatty acid in combination with intermittent calorie restriction on the development of mammary tumors. Dr. Grossmann has also initiated a series of in vitro studies to assess the effects of several long chain fatty acids on the interaction of cell proliferation and death and is seeking funding to evaluate the chemopreventive effects of these compounds in preclinical models.

We have also investigated the effects of intermittent restriction intervention in a model for prostate cancer, TRAMP mice. This intervention also protected against prostate cancer development as reflected by a delay in the initial detection of the disease as well as a later age at death. Furthermore, the intermittent restriction appears to be far superior to chronic calorie restriction which had little effect on prevention of prostate cancer in TRAMP mice. We are also trying to assess the effects of obesity on the development of prostate cancer. Our initial goal was to study the effects of obesity initiated at different ages on the development of prostate cancer using the TRAMP model. However, there have been technical difficulties with inducing the obesity with the chemical agent that we have now tried several alternative approaches. We have recently completed diet-induced obesity study in the TRAMP mice. The results of these studies suggest that, as in humans, obesity is associated with more severe disease. We have also completed a study implanting obese mice with a TRAMP prostate cancer cell line and are evaluating those results. In these studies, we will assess the roles of leptin and adiponectin in the development of prostate cancer.

Left to right front row: Soner Dogan, Kelsey Kittleson (Intern), Margot Cleary, Nancy Mizuno, Emily Kain Quealy

Back row:Katai Nkhata, Christine Seppanen, Michael Grossmann, Olga Rogozina

Collaborations continue with Dr. Nita Maihle at Yale University and Dr. Joseph Grande from the Mayo Clinic. Dr. Maihle has provided molecular biology support and much encouragement. Dr. Grande is instrumental in providing histopathology analyses and interpretation. We also have collaboration with Dr. Anna Loshkin from the University of Pittsburgh Cancer Center for serum analyses. We are also providing tissues from our experiment animals to other investigators at the University of Minnesota. We have undertaken several studies with Dr. Johnny Lü. Other internal collaborations are ongoing with Dr. Joshua Liao and Dr. Rick Brown. The intensive efforts to undertake these studies and develop a new and exciting area of research in our laboratory has reached the point where we are recognized as pioneers in this area, as evidenced by the papers and grants I am asked to review over the past few years, invitations to present our data at meetings and at other institutions, and requests to write review articles.

Other Professional Activities
Margot P. Cleary

Invited speaker:
- Third Hormel Institute Frontiers in Cancer Research, Austin, MN
- 39th International Symposium of the Princess Takamatsu Cancer Research Fund, Tokyo, Japan

Grant reviewer:
- Permanent Member NIH review panel Chemo/Dietary Prevention
- Italian Association of Cancer Research
- NIH Special Emphasis Panel

CELL DEATH AND CANCER GENETICS

Section Leader

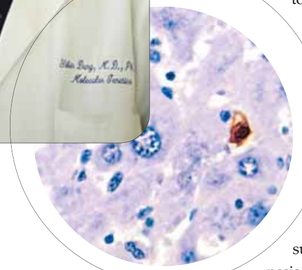
Yibin Deng, M.D., Ph.D.

Assistant Professor

We began establishing our new Hormel Institute laboratory in May 2009. The focus of our research is to understand how wild-type p53 controls apoptosis, senescence, and autophagy in pre-cancerous cells and cancerous cells, and how mutant p53 identified in cancer patients disrupts these processes and impacts tumor initiation, progression, metastasis and cancer therapy in vivo. Our long-term goal is to identify the genetic pathways that contribute to p53-mediated tumor suppression in vivo and utilize the genetics of p53 pathway components in cancer cells to improve current cancer therapies and develop novel molecular targets for human cancers. Our approach emphasizes genetics, and we are increasingly relying on new types of genetically engineered mouse models that faithfully recapitulate the salient features of human cancer to explore the key signaling pathways that allow cancer cells to escape p53-mediated checkpoints. The research in our laboratory can be divided into 3 major areas:

1. Modeling human breast cancer in mouse

Recent systematic analyses have revealed that the most common genetic alterations in human breast cancers are mutation of the tumor suppressor gene p53. Furthermore, molecular epidemiology studies indicate that p53 mutations are strongly associated with poor prognosis, tumor recurrence, and chemo- and radiation-therapy resistance in breast cancer patients. Given the crucial function of p53 in inhibiting breast tumor development in humans, it is very critical to establish a mouse model with p53 mutation that faithfully represents the same types of disease states seen in human breast cancers driven by the same genomic lesions. However, the failure of the p53 null mutation mouse to fully mimic the characteristics of human breast cancers could be attributed to the fact that p53 null mutation is rarely found in breast cancer patients. Contrarily, most of human breast tumors have a missense mutation in the p53 gene. Therefore, generation of a mouse model with a p53 missense mutation rather than a p53 null mutation in mammary gland tissue may more faithfully induce tumors that represent the characteristics observed in those found in breast cancer patients. We are using the strength of both conditional wild-type p53 knock-out and mutant p53 knock-in mice to generate a mouse breast cancer model with a p53 "hot spot" mutation (arginine (R) at position 172 to histidine (H), equivalent to human R175H seen in breast cancer patients) at physiological levels in mouse mammary glands. Within this model, we will be able to analyze the relevant genetic pathways involved in breast tumor development and target these pathways with novel therapeutics to potentially suppress mammary tumorigenesis.



2. p53-induced autophagy and tumorigenesis

Apoptosis is a genetically controlled form of cell death that is important for normal development and tissue homeostasis. Senescence produces "genetic death" in that the senescent cell is incapable of further propagation. While the induction of apoptosis and senescence has been recognized as the key mechanism by which p53 eliminates cancer cells, the contribution of p53-mediated autophagy, a highly regulated membrane-trafficking process leading to bulk turnover of cellular proteins and intracellular organelles in lysosome, to prevent or stall tumor development, is now being appreciated. We recently generated a conditional DRAM (Damage Regulated Autophagy Modulator, a p53 downstream target gene) knock-out mouse model to test our hypothesis that DRAM-initiated autophagy plays a crucial role in suppressing tumorigenesis in vivo. Our laboratory is using the conditional DRAM knock-out mice to breed with p53-mediated apoptosis deficient mice (p53R172Pknock-in) and senescence-deficient mice (p21 knockout) to generate the "triple" mutant mice dissecting the critical function of p53-mediated autophagy, apoptosis and senescence in suppressing spontaneous cancer development in vivo. Our study will ultimately lead us to identify the genetic pathways and the molecular targets involved in autophagy, apoptosis, and senescence that may be perturbed in human cancerous cells and to understand how these pathways impact on cancer initiation, progression, metastasis, and chemotherapeutics.

Left to right: Jian Kang Deng, Xiaolan Guo, Yibin Deng

3. Exploring the molecular and cellular basis of drug sensitivity and resistance

Our laboratory has a long-standing interest in understanding genetic pathways that allow targeting cancer cells while leaving normal cells untouched. 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 key pathways that are linked to chemotherapy resistance with the long-term goal of identifying new targets to specifically kill cancerous cells and combat acquired drug resistance.

CELLULAR DYNAMICS

Section Leader

Edward H. Hinchcliffe, Ph.D.

Associate Professor

Our section, which is new to The Hormel Institute as of December 2008, is currently funded by an R01 research grant from the National Institutes of General Medicine to study the molecular mechanisms that regulate centrosome reproduction and function. The centrosome is an organelle that nucleates and organizes the microtubule cytoskeleton. This in turn is used to build the mitotic spindle, which is responsible from aligning and segregating the duplicated chromosomes during cell division. Centrosomes are thought to play a major role in establishing the polarity of the mitotic spindle – which must be bipolar in order to ensure that the cell divides into exactly two daughter cells. To maintain this bipolarity, the single centrosome normally duplicates exactly once during the cell cycle, yielding two centrosomes that can form two spindle poles. In certain advanced cancer cells, the number of centrosomes increases to greater than two, with a commensurate increase in spindle pole number and an increase in the probability of abnormal cell division. Therefore, it is important to understand the molecular mechanisms that drive normal centrosome duplication, and importantly, restrict centrosome duplication to once per cell cycle. In the Hinchcliffe lab we use cultured mammalian cells and cytoplasmic extracts generated from Xenopus frogs to examine the basic control mechanisms underlying centrosome duplication. 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. We are currently

Experimental research results

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, theses 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 the number of nuclear-associated centrosomal foci does not. 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. This study has been completed and was submitted for publication in May, 2008.

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 (and do) 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 and astral microtubules at the spindle poles during mitosis.

The results of a previous study (Hinchcliffe et al., 2001 Science 291:1547-50) revealed that the centrosome also plays a regulatory role in cell cycle progression. We removed the centrosome from living tissue culture cells by microsurgery and followed the resultant acentrosomal cells (termed karyoplasts) by time-lapse videomicroscopy. We found that karyoplasts show three major defects in their cell cycle: (i) duration of mitosis is three times as long as control cells; (ii) karyoplasts always initiate cytokinesis in a bipolar fashion, but ~40% of the time they fail cleavage and exit mitosis as a single multinucleate cell; (iii) post-mitotic karyoplasts –regardless of whether or not they cleaved - arrest cell cycle progression prior to the onset of the next S-phase. Together, these findings suggest that the centrosome plays a crucial role in ensuring that events of the cell cycle occur in a timely fashion.

Left to right:
Edward Hinchcliffe, Kul Karanjeet

We are currently 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 BSC-1 cells that constitutively express α-tubulin coupled to-GFP. We are using these cells to investigate the role of the centrosome in establishing the polarity of the mitotic spindle. The results of our studies have revealed that acentrosomal cells either separate their acentrosomal microtubule organizing center, thereby generating a bipolar spindle, or fail to do so, and enter mitosis with a persistent monopolar spindle. In the case of the bipolar mitosis, the cells divide with normal timing. However, in the ~40% of cells that enter mitosis with a monopolar spindle, the cells cannot bipolarize, and fail cytokinesis. This work reveals that centrosomes are absolutely necessary in order to establish a strictly bipolar spindle morphology during mitosis.

A detailed understanding of the regulation of cell division will advance our knowledge of the biology of cancer – itself a disease characterized by unregulated cell proliferation. Much of our current knowledge on the molecular regulation of cell division has come from genetic studies in yeast and this holds true for the role of the centrosome in these processes (the centrosome in yeast is referred to as the spindle pole body). However, it remains an important task to understand how centrosome-dependent mechanisms function in mammalian cells. It is reasonable to expect that studying experimentally derived acentrosomal mammalian cells will provide unique and important insights into the molecular mechanisms that regulate the cell division cycle in intact cells. Thus, our experiments will compliment the genetic and biochemical work currently being done in yeast and other organisms. Together, these studies should provide a fertile source of potential targets for future anti-cancer drugs.

Results of our research have most recently been published in Journal of Cell Biology, Journal of Cellular Physiology, Cell Motility and the Cytoskeleton, Infection and Immunity, and Seminars in Cell and Developmental Biology.

TRANSLATIONAL CANCER RESEARCH

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

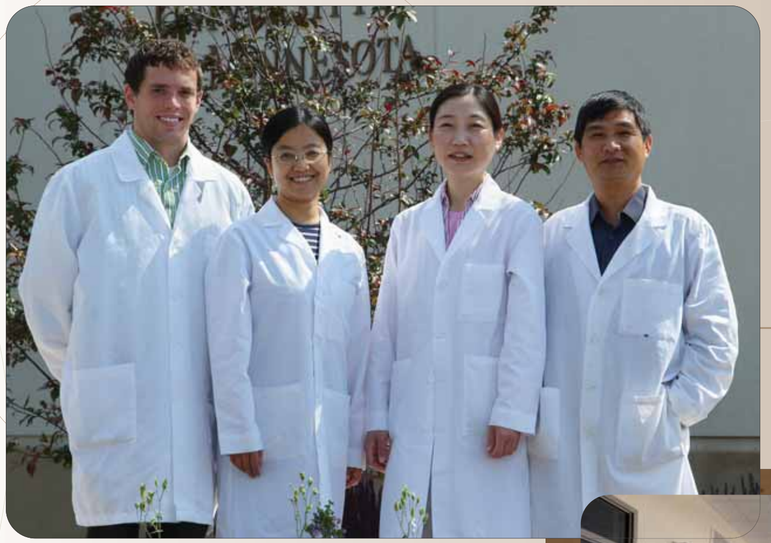
In the past two years or so, we have been shifting our research focus from molecular carcinogenesis to the effects of RNA processing on cancer progression and chemoresistance, mainly because RNA processing has been a fast developing area in basic biology and its potential in the understanding of cancer development and progression is anticipated to be huge. While genomic alterations (i.e. changes in DNA) in cancer cells have been studied for nearly a century, alterations in RNA processing, collectively referred to as ribonomics, have just been studied recently in cancer cells. RNA processes include alternative splicing, RNA editing, quality control decay, and biogenesis of siRNA, microRNA and other small or large non-coding RNA. Our laboratory currently focuses on how aberrant expression of c-myc and cyclin D1 affects alternative splicing of certain oncogenes and tumor suppressor genes, which in turn influences progression of breast and pancreatic cancer cells to metastatic or chemoresistant status. We also study how c-myc and cyclin D1 regulate expression of certain microRNA to influence formation of breast and pancreatic cancers. By manipulating c-myc or cyclin D1 regulated alternative splicing, we hope to develop some novel strategies or agents for chemotherapy of advanced breast and pancreatic cancers.

D1-cdk4 chemical inhibitors

We synthesized eight novel indolocarbazole analogs as cdk4 inhibitors. Some of them show high therapeutic efficacy for breast and pancreatic cancer cell lines in culture, with IC50 less than 1 μM. Preliminary mechanistic studies performed on the parental compound named NPCD show that it effects by decreasing cyclin-dependent kinase inhibitor p27 and p21 in all the cells tested. NPCD also decreasing cyclin D1 in some, but not all, cancer cell lines. As a net result, phosphorylation of Rb protein is inhibited, leading to cell cycle arrest and death of cancer cells.

RSK4 project

Our previous studies show that RSK4 at the full-length sequence may be a potent inhibitor of breast cancer invasion and metastasis. However, in the past years, we cloned the 5′-end of human and mouse RSK4 and identified two initiation sites, from which RSK4 is transcribed to two mRNA variants, i.e. RSK4α and RSK4ß. We also identified two alternative splicing sites of RSK4. Two different pre-mRNA and two splicing sites together produce eight mRNA variants, and our preliminary studies show that different RSK4 variants actually have different and even opposite functions. We now have evidence showing that while the full-length RSK4 is an inhibitor of breast cancer invasion and metastasis, the shortest variant that lacks the whole exon 25 (exon 24 in the mouse) and part of the exon 23 (exon 22 in the mouse) may promote cell growth and inhibit cell death. It is likely that RSK4 is regulated via alternative splicing during cancer cell progression and chemotherapy to gain a better survival advantage. Therefore, targeting this alternative splicing to direct it toward the formation of the full-length RSK4 may be a good strategy for cancer treatment.



Left to right: Timothy Finley (Intern), Min Yang, Yuan Sun, Joshua Liao

STEM CELLS AND CANCER

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

Research in my laboratory has for the past twenty-six years focused on the identification and characterization of keratinocyte stem cells and evidence for their role as skin tumor-initiating cells. These studies culminate in the development of our Krt1-15CrePR1;R26R transgenic mice that enable striking visualization of tumor formation from hair follicle stem cells. During the next five years, we will continue to refine the phenotype of the keratinocyte stem cells, to determine the role of bone marrow-derived cells in skin cancer development, and to understand regulation of keratinocyte stem cell division.

I will use our novel transgenic mouse model, Krt1-15CrePR1;R26R, to determine the contribution of hair follicle bulge stem cells to cutaneous neoplasms in mice resulting from skin exposure to ultraviolet light. We will employ two strategies to attack this problem. First, we will determine the contribution of Krt1-15-expressing hair follicle stem cells to tumors when irradiated with ultraviolet light. We will transiently induce the Cre recombinase before irradiation. Upon induction, the Cre recombinase will recognize its loxP sites in the disrupted, ubiquitously expressed transgene for beta-galactosidase and reconstitute an intact, beta-galactosidase that can be histochemically stained blue. Prior to irradiation, the blue cells will be found only in the hair follicle bulge at the site of Krt1-15-expression. During the course of irradiation, we will determine whether the blue cells that are the progeny of the Krt1-15-expressing cells are found in the developing papillomas and carcinomas. Second, we will determine whether the blue-stained cell vs. unstained cell components of the resulting tumors bear signature mutations in the p53 gene. This approach will employ laser capture microdissection of blue-stained and unstained regions of the tumors followed by DNA isolation, PCR amplification of regions bearing possible signature mutations, and sequencing of those regions. We anticipate that keratinocyte stem cells from the hair follicle bulge will play a significant role in the pathogenesis of UVB-induced skin tumors. Based on ongoing studies using the chemically induced model of skin cancer, we expect that hair follicle stem cells may not be the only targets in skin cancer. We expect these proposed studies will lead to novel insight into the origins and pathogenesis of non-melanoma skin cancer.

We will also determine the role of bone marrow derived cells in skin cancer development. In the multistage model of cutaneous carcinogenesis, a single subtumorigenic exposure to a carcinogen (initiation) and subsequent chronic regenerative epidermal hyperplasia of sufficient magnitude (promotion) can induce benign and malignant neoplasms of the cutaneous epithelium. Tumor initiation is thought to convert selected epithelial cells into latent neoplastic cells, whereas promotion elicits expression of the neoplastic change leading to tumor formation. Although such cancers are widely believed to originate from the transformation of epidermal or hair follicle stem cells, the recent demonstration that gastric cancer originates from bone marrow-derived cells challenges this dogma. A further objective of this investigation is to determine the relative contributions of hair follicle stem cells and bone marrow derived cells (BMDC) to skin tumors. Our hypothesis is that the chronic tissue damage characteristic of skin tumor promotion recruits BMDC into ensuing papillomas and carcinomas. First, we will determine the relative contributions of BMDC and hair follicle stem cells in the formation of skin tumors. Our method of approach will be to transplant genetically labeled BMDC to lethally irradiated Krt1-15CrePR1;R26R recipients subjected to chemically-induced skin cancer and where the Cre-recombinase is induced before exposure to the carcinogen. We will then assess developing epithelial skin lesions for cells of donor origin (expressing EFGP and the Y chromosome) vs. hair follicle stem cell origin (blue X-gal staining cells) in skin sections. Second, we will determine in the Krt1-15CrePR1;R26R transgenic mice engrafted with genetically marked BMDCs whether labeled keratinocytes from the Krt1-15-expressing stem cells from the hair follicle bulge vs. those keratinocytes derived from genetically marked BMDC express the signature A>T mutation in codon 61 of the Ha-ras gene. At intervals during skin tumor promotion, we will use Laser Capture Microdissection followed by mutation analysis to compare the tumor Krt1-15 progeny vs. BMDCs-derived keratinocytes to determine the presence of the initiated lesion. These experiments will determine the contributions of BMDCs and will have significant implications for the multistage model of skin cancer and for the development of anticancer therapies in that nonmelanoma skin cancer, and indeed other epithelial cancers, may need to be viewed as systemic rather than local diseases.

Left to right: Nyssa Readio, Rebecca Morris, Ashok Singh, Meepa Lokuge

Our third new area of research is to identify specific 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 disease. Hence, identification of genes regulating their number and proliferative potential is a critical problem in cutaneous biology. We propose here a novel strategy for identifying genes involved in keratinocyte stem cell regulation. This strategy takes advantage of several recent important advances made in our laboratory: 1) identification of a selectable marker of 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 of the proposed research is to identify major genes regulating the number of keratinocyte stem cells. Our hypothesis is that there are specific genes and pathways that regulate the number of keratinocyte stem cells that may be different from those regulating transit-amplifying cells. We first plan to use genetic tools to refine the linkage intervals we have already identified. Next, we will use a candidate gene approach for stem cell gene identification. Finally, we will use a complementary global genomic approach to identify associated molecular pathways, and to assess regulatory polymorphisms causing differences in gene expression in the absence of coding sequence differences within the quantitative trait locus. This investigation will enable the identification of major genes and regulatory pathways in keratinocyte stem cells. This research will impact the fields of cutaneous biology and stem cell research and should provide new insight into the mechanism of skin 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, for wounds that do not heal, and for skin cancer.

SIGNAL TRANSDUCTION AND APOPTOSIS

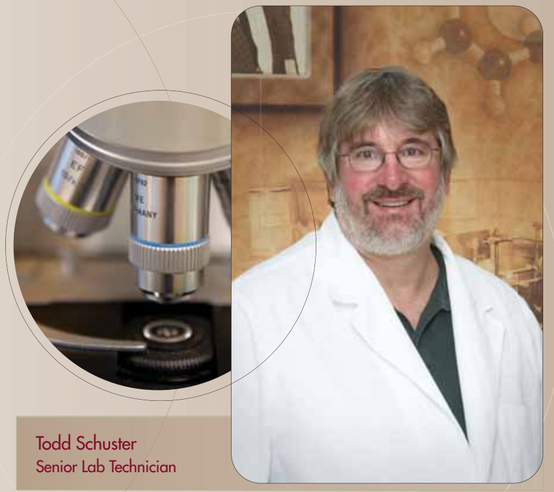
Section Leader
Peter Ruvolo, Ph.D.
Associate Professor

Currently in its third year of operation, our section continues to focus on identifying the various cell signaling pathways that regulate cell death and ultimately influence tumor development and drug resistance in cancers such as leukemia and lung cancer. Our research continues to focus on signal transduction pathways that regulate chemoresistance and tumorigenesis in leukemia. Although acute lymphoblastic leukemia (ALL) is the most common malignancy among children and boasts cure rates ~ 80%, 20% of these children continue to relapse each year making relapsed ALL the 5th most common cancer in pediatrics and the second most common cause of death due to a disease. Novel agents and/or improved therapies are therefore greatly needed for this population of children. Recently, we determined that the dsRNA dependent Protein Kinase (PKR) is basally active in ALL cell lines. This finding is unexpected as PKR was believed to be activated only during stress. In the B-precursor ALL cell line REH, PKR was found to activate the pro-survival kinase AKT. On the other hand, PKR is necessary for drug-induced cell death in REH cells. These findings suggest that PKR may promote cell survival in ALL cells as well as be essential for cell death in response to therapy. Studies are underway in collaboration with Dr. Michael Burke of the University of Minnesota's Masonic Cancer Center and Department of Pediatrics to determine if PKR is a prognostic factor according to disease risk group and/or response to therapy in pediatric ALL. Initial data has indicated that PKR is indeed active in ALL blast cells from patients and thus PKR may prove to be a target for treating ALL.

Our laboratory previously determined that the BCL2 phosphatase was activated by ceramide and involved a mitochondrial PP2A isoform. Recent studies in our laboratory have found that ceramide activates a PP2A isoform that dephosphorylates BCL-XL (another BCL2 family member that has been implicated as a tumor promoter) in lung cancer cells. Ceramide was found to promote mitochondrial PP2A activity in lung cancer cells. Furthermore, suppression of PP2A catalytic subunit by siRNA promoted BCL-XL phosphorylation indicating for the first time that PP2A is indeed a BCL-XL phosphatase. The mechanism how phosphorylation of BCL-XL regulates function is controversial since some studies suggest phosphorylation supports function and others suggest phosphorylation impedes function. Like BCL2, Taxol-induced phosphorylation of BCL-XL suppresses its anti-apoptotic function. However, previous studies from our group have shown that BCL-XL is basally phosphorylated in lung cancer cells suggesting that phosphorylation does not necessarily inactivate function. In the past year, we have found that BCL-XL phosphorylation at serine 62 is necessary for the molecule's full and potent anti-apoptotic function. Ceramide was found to suppress phosphorylation of BCL-XL suggesting that dephosphorylation of the molecule might be important in the death process. It was determined that the mitogen activated protein kinase ERK phosphorylates BCL-XL at serine 62. This was a novel finding as a survival kinase for BCL-XL has yet to be identified. A mechanism how phosphorylation supports survival function appears to involve p53, which binds to and inhibits BCL-XL function. Supporting this model, inhibition of BCL-XL phosphorylation promoted association with p53. The findings from these studies suggest new targets for the treatment of lung cancer where we can suppress ERK-mediated phosphorylation of BCL-XL or promote PP2A-mediated dephosphorylation of the molecule to suppress BCL-XL's survival function.

Left to right: Vivian Ruvolo, Peter Ruvolo

Work continues on a collaborative project with Michael Andreeff at MD Anderson Cancer Center (Houston, TX) to characterize the role of BCL2 family members in acute myeloid leukemia (ALL). This project is part of a Program Project funded by the National Cancer Institute entitled "Therapy of AML." In recent studies we have found that the fenretinide, a drug that is being used clinically to treat leukemia and a number of solid tumors, may promote cell death by mechanisms involving the BCL family of proteins, heat shock proteins, and protein kinase C.



Todd Schuster
Senior Lab Technician

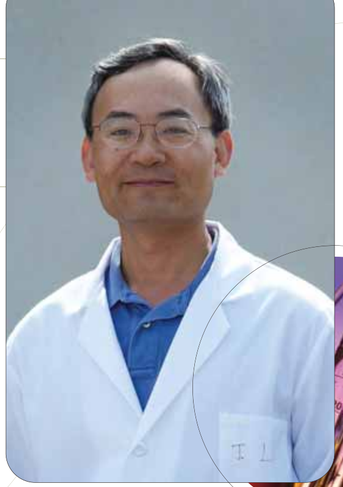
Schuster operates, maintains and instructs scientists about the shared instruments used at The Hormel Institute for cancer research including FACs cell sorter, FACs calibur flow cytometer, real time PCR, and confocal and fluorescent microscopes.

CANCER BIOLOGY

Section Leader

Junxuan (Johnny) Lü, Ph.D.

Professor



Our section's long-term goals are to understand the biochemical, cellular and molecular processes crucial for the genesis of cancer and to develop mechanism-based cancer prevention and therapeutic strategies for implementation through supplements, functional & medicinal foods or drug approaches. Our research program has continued to focus on the following two areas:

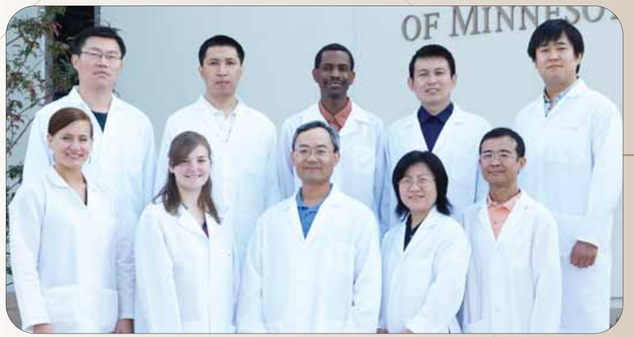
- sustaining our research excellence in understanding the cellular and molecular mechanisms by which the trace element nutrient selenium in cancer chemoprevention and treatment.
- identifying and developing novel cancer chemopreventive and therapeutic agents based on Chinese and Oriental medicinal herbs.

Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit

Chemoprevention of prostate cancer by second-generation selenium compounds in reference to selenomethionine holds strong promise to deal with the disease at the root. We used the transgenic adenocarcinoma mouse prostate (TRAMP) model to establish the efficacy of methylseleninic acid (MSeA) and methylselenocysteine (MSeC) against prostate carcinogenesis and to characterize potential mechanisms. Eight-week-old male TRAMP mice (C57B/6 background) were given a daily oral dose of water, MSeA, or MSeC at 3 mg Se/kg body weight and were euthanized at either 18 or 26 weeks of age. By 18 weeks of age, the genitourinary tract and dorsolateral prostate weights for the MSeA- and MSeC-treated groups were lower than for the control ($P < 0.01$). At 26 weeks, 4 of 10 control mice had genitourinary weight >2 g, and only 1 of 10 in each of the Se groups did. The efficacy was accompanied by delayed lesion progression, increased apoptosis, and decreased proliferation without appreciable changes of T-antigen expression in the dorsolateral prostate of Se-treated mice and decreased serum insulin-like growth factor I when compared with control mice. In another experiment, giving MSeA to TRAMP mice from 10 or 16 weeks of age increased their survival to 50 weeks of age, and delayed the death due to synaptophysin-positive neuroendocrine carcinomas and synaptophysin-negative prostate lesions and seminal vesicle hypertrophy. Wild-type mice receiving MSeA from 10 weeks did not exhibit decreased body weight or genitourinary weight or increased serum alanine aminotransferase compared with the control mice. Therefore, these selenium compounds may effectively inhibit this model of prostate cancer carcinogenesis.

In vivo molecular mediators of cancer growth suppression and apoptosis by selenium in mammary and prostate models: lack of involvement of gadd genes

We used acute selenium (Se) treatments (i.e., daily single oral gavage of 2 mg Se per kilogram of body weight for 3 days) of female Sprague-Dawley rats bearing 1-methyl-1-nitrosourea-induced mammary carcinomas to increase the probability of detecting in vivo apoptosis and the associated gene/protein changes in the cancerous epithelial cells. The results show that whereas control carcinomas doubled in volume in 3 days, Se-methylselenocysteine and selenite treatments regressed approximately half of the carcinomas, accompanied by a 3- to 4-fold increase of morphologically observable apoptosis and approximately 40% inhibition of 5-bromo-2'-deoxyuridine index of the cancerous epithelial cells. The mRNA levels of growth arrest-DNA damage inducible 34 (gadd34), gadd45, and gadd153 genes were, contrary to expectation, not higher in the Se-treated carcinomas than in the gavage or diet restriction control groups. The gadd34 and gadd153 proteins were localized in the nonepithelial cells and not induced in the cancer epithelial cells of the Se-treated carcinomas. On the other hand, both Se forms decreased the expression of cyclin D1 and increased levels of P27Kip1 and c-Jun NH2-terminal kinase activation in a majority of the mammary carcinomas. Furthermore, the lack of induction of gadd genes in vivo by methylseleninic acid was confirmed in a human prostate xenograft model in athymic nude mice. In summary, these experiments showed the induction of cancer epithelial cell apoptosis and inhibition of cell proliferation by Se in vivo through the potential involvement of cyclin D1, P27Kip1, and c-Jun NH2-terminal kinase pathways. They cast doubt on the three gadd genes as mediators of Se action in vivo.



Front row left to right: Emily Kain Quealy, Jennifer Walsh (Intern), Johnny Lu, Li Li, Yubo Chai

Back row: Jinhui Zhang, Yong Zhang, Katai Nkhata, Lei Wang, Kwan Hyun Kim

These studies, as well as our previous studies, further support the methylselenium compounds as potential candidate second generation selenium agents for translational investigation in the wake of the failed SELECT (selenium vitamin E chemoprevention trial) study that found no preventive effect of selenomethionine in men against prostate cancer.

Identification of novel cancer chemopreventive and therapeutic agents

We have documented efficacy of Penta-1,2,3,4,6-O-galloyl-beta-D-glucose (PGG) against prostate cancer growth and elucidated potential in vitro mechanisms. The data supported a 5-year grant award from NCI for further evaluating the merit of PGG for prostate cancer prevention. We also reported the in vivo anti-cancer efficacy of herbal extract from Korean Angelica and its major compound decursin. The published work on anti-androgen compounds from herbal sources supported a provisional patent application by the U of M OTC.

Penta-1,2,3,4,6-O-galloyl-beta-D-glucose induces p53 and inhibits STAT3 in prostate cancer cells in vitro and suppresses prostate xenograft tumor growth in vivo

Penta-1,2,3,4,6-O-galloyl-beta-D-glucose (PGG) is a naturally occurring gallotannin from some Oriental herbs. Several cell culture studies suggested a potential for PGG as a novel agent for the chemoprevention and treatment of cancer. We investigated the cell death signaling mechanisms induced by PGG in human prostate cancer cells of different p53 functional status. We observed the induction of G(1)- and S-phase arrests and caspase-mediated apoptosis in the androgen-dependent human LNCaP cells, which express wild-type p53, and in the androgen-independent, p53-mutant DU145 cells. In LNCaP cells, caspase-mediated apoptosis induction by PGG was associated with and mediated in major part by activation of p53 as established through small interfering RNA knockdown and dominant-negative mutant approaches. Intracellular reactive oxygen species production by PGG was found to be crucial for these molecular and cellular actions. In DU145 cells, which harbor constitutively active signal transducer and activator of transcription 3 (STAT3), caspase-mediated apoptosis induction by PGG was associated with an inhibition of STAT3 Tyr705 phosphorylation and the down-regulation of STAT3 transcriptional targets Bcl-XL and Mcl-1. Overexpression of Bcl-XL or knockdown of its binding partner Bak attenuated apoptosis induction. Furthermore, we provide, for the first time, in vivo data that PGG significantly inhibited DU145 xenograft growth in an athymic nude mouse model in association with an inhibition of pSTAT3. Our data support PGG as a multitargeting agent for chemoprevention and therapy of prostate cancer by activating the p53 tumor suppressor pathway and by inhibiting STAT3 oncogenic signaling.

Penta-O-galloyl-beta-D-glucose induces S- and G(1)-cell cycle arrests in prostate cancer cells targeting DNA replication and cyclin D1

We have recently shown that penta-1,2,3,4,6-O-galloyl-beta-D-glucose (PGG), a naturally occurring hydrolyzable gallotannin, inhibited the in vivo growth of human androgen-independent p53-mutant DU145 prostate cancer (PCa) xenograft in athymic nude mice without adverse effect on their body weight. We have also shown that PGG induced caspase-mediated apoptosis in the DU145 cells and the androgen-dependent human p53-wild-type LNCaP cells. Here, we investigated the cell cycle effects of PGG in these and other PCa cells. Our data show that treatment with subapoptotic doses of PGG induced S-arrest, whereas higher doses of PGG induced not only S-arrest but also G(1) arrest. We show, for the first time, that irrespective of the p53 functional status of the PCa cell lines, PGG exerted a rapid (within 2 h) and potent inhibition (inhibitory concentration by 50% approximately 6 microM) of 5-bromo-2'-deoxyuridine incorpo-

ration into S phase cells. In isolated nuclei, PGG inhibited DNA replicative synthesis with superior efficacy than a known DNA polymerase alpha inhibitor, aphidocolin. In addition to the S-arrest action, we have found a close association of downregulation of cyclin D1 with G(1) arrest induced by PGG. Overexpressing this G(1) cyclin abolished G(1) arrest, but hastened the S-arrest induction by PGG. Together, our data indicate that PGG induced PCa S-arrest probably through DNA replicative blockage and induced G(1) arrest via cyclin D1 downregulation to contribute to anticancer activity. Our data raise the hypothesis that PGG may be a novel inhibitor of DNA polymerases.

In vivo anti-cancer activity of Korean Angelica gigas and its major pyranocoumarin decursin

We have reported that a 10-herbal traditional formula containing Korean Angelica gigas Nakai (AGN) exerts potent anti-cancer efficacy and identified decursin and decursinol angelate (DA) from AGN as novel anti-androgens. We determined whether AGN would exert in vivo anti-cancer activity and whether decursin or DA could account for its efficacy. The AGN ethanol extract was tested against the growth of mouse Lewis lung cancer (LLC) allograft in syngenic mice or human PC-3 and DU145 prostate cancer xenograft in immunodeficient mice. The pharmacokinetics of decursin and DA were determined. The AGN extract significantly inhibited LLC allograft growth (30 mg/kg) and PC-3 and DU145 xenograft growth (100 mg/kg) without affecting the body weight of the host mice. Biomarker analyses revealed decreased cell proliferation (Ki67, PCNA), decreased angiogenesis (VEGF, microvessel density) and increased apoptosis (TUNEL, cPARP) in treated tumors. Decursin and DA injected intraperitoneally were rapidly hydrolyzed to decursinol. Decursinol and decursin at 50 mg/kg inhibited LLC allograft growth to the same extent, comparable to 30 mg AGN/kg. Therefore the AGN extract possessed significant in vivo anti-cancer activity, but decursin and DA only contributed moderately to that activity, most likely through decursinol.

Anti-androgen receptor signaling and prostate cancer inhibitory effects of sucrose- and benzophenone-compounds

Novel agents that target multiple aspects of androgen receptor (AR) signaling are desirable for chemoprevention and treatment of prostate cancer (PCa). We aimed to identify compounds isolated from medicinal herbs as such drug candidates. In the LNCaP human androgen sensitive PCa cell model, we tested five compounds purified from Lindera fruticosa Hemsley in the range of 10-50 microM for growth inhibition and AR-prostate specific antigen (PSA) suppressing potency. We determined the relationship between these activities and P53 tumor suppressor protein activation and apoptotic cleavage of PARP. We compared these compounds to the anti-androgen drug Casodex/bicalutamide to identify mechanistic novelty. Among 3 sucrose compounds, beta-D-(3,4-di-sinapoyl)fructofuranosyl-alpha-D-(6-sinapoyl)glucopyranoside decreased AR and PSA mRNA and protein levels in LNCaP cells and inhibited androgen-stimulated AR translocation from the cytosol to the nucleus. This compound also increased P53 Ser(15) phosphorylation and PARP cleavage in LNCaP cells, but required higher dosage than for suppressing AR-PSA. Interestingly, this compound did not inhibit the growth of RWPE-1 non-transformed prostate epithelial cells. The benzophenone compound 2-methoxy-3,4-(methylenedioxy)benzophenone suppressed PSA and AR in LNCaP cells without apoptosis. Our data support novel anti-AR actions of these herbal compounds distinct from Casodex and merit further investigation as drug candidates.

Collaborations

- Sung-Hoon Kim, Kyunghee University, Korea, to identify and develop novel agents for prostate cancer prevention based on Oriental medicine
- Margot Cleary, Section of Nutrition and Metabolism, on selenium as a preventive agent for prostate cancer with the TRAMP model
- Chengguo Xing, U of M Department of Medicinal Chemistry, for synthesis of decursin-derivatives and isolation of penta galloyl glucose from tannic acid
- Gary Piazza, Southern Research Institute, Birmingham, AL on the prostate cancer chemoprevention by novel sulindac derivatives devoid of COX-2 inhibitor activity

Other Professional Activities
Junxuan (Johnny) Lü

Invited speaker:

- Plenary presentation "Cancer Prevention by Selenium"
  International Symposium on Novel Strategies for Targeted Prevention and Treatment of Cancer, Jawaharlal Nehru University, New Delhi, India.
- "Anti-cancer Herbal Cocktails and Active Compounds"
  Third Hormel Institute Symposium/8th International Skin Carcinogenesis Conference, Austin, MN.
- Plenary Presentation "Prostate Cancer Chemoprevention by Novel Oriental Herbal Agents" KyungHee University CPMDRC International Symposium, Seoul, Korea.
- Plenary presentation "Cancer Chemoprevention by Selenium"
  KyungHee University workshop on selenium, Seoul, Korea.

Grant reviewer:

- NIH 2009/10 ZRG1 OTC-K (58) RFA OD-09-003 Challenge Grants Panel 10

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 10 research sections for its many ongoing research projects. Each of the institute's cancer research departments is dedicated to preventing or controlling cancer and the RSG department provides critical support to project operations.

Left to right: Alyssa Vrieze, Teri Johnson, Ellen Kroc, Michelle Jacobson
Not pictured: Andria Hansen

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.

Office Staff

Tonya Poorman, Principal Office and Administrative Specialist
Betsy Mentel, Executive Office and Administrative Specialist
Becky Smit, Principal Accounts Specialist
Kimberly Houghtaling, Principal Accounts Specialist

Left to right: Tonya Poorman, Betsy Mentel, Dr. Ann M. Bode, Becky Smit
Not pictured: Kimberly Houghtaling

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.

PUBLIC RELATIONS AND DEVELOPMENT
Gail Dennison

The Public Relations and Development office of The Hormel Institute experienced another fast-paced year, managing communications, marketing, fundraising development, promotional events and visual communication projects related to the major expansion. The Hormel Institute's visibility grew exponentially in the past year because of the completion of the beautiful world class research building and the continually significant research achievements of its faculty. The growing partnerships with Mayo Clinic, University of Minnesota-Rochester and IBM made 2008-2009 a year when progress was highly visible.

Visual communication elements were an exciting part of the expansion with a capstone project the beautiful wall mural in the institute's new lobby. The montage, capturing the story and history of The Hormel Institute, was created by Mayo Clinic graphic artists and produced/installed by Hormel Foods' Studio H.

The Grand Opening of The Hormel Institute Expansion Project was launched October 3, 2008 and continued throughout the month. A three-day world cancer research conference was held in Austin October 4-7 as part of the celebration, bringing new growth/economic benefits to our community.

We remain deeply thankful for the support we consistently and generously receive from our community, our elected officials and our regional partners:

The Hormel Foundation
Hormel Foods Corporation
Mayo Clinic Rochester
Representative Tim Walz
Senator Amy Klobuchar
Development Corporation of Austin
Southern Minnesota Initiative Foundation
Austin Area Chamber of Commerce
City of Austin
Mower County
Austin Area Foundation
University of Minnesota-Rochester
Riverland Community College
IBM Rochester
GRAUC – Greater Rochester Advocates for Universities and Colleges
BioBusiness Alliance of Minnesota
LifeScience Alley

Research Support Services (RSS)
Distance Outreach and Education (DOE)
Supervisor: Craig Jones

It has been another exciting year for RSS. The Blue Gene supercomputing project is continuing and has been expanded to include two 3D modeling systems located in the large conference room and the seminar room. These systems use polarized light with inexpensive glasses facilitating detailed visualization for larger groups. We have also added a couple more general purpose molecular modeling workstations. Of course, we continue to provide instrument maintenance, 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 work load.

Left to right, front row: Ryan Wiersma, Tim Lastine, Theresa Tucker, Rose Srock, Craig Jones, Mike Conway.

Our DOE program, in cooperation with The Southern Minnesota Internet Group (SMIG), is making technology available to many rural citizens throughout a large area of Southern Minnesota. The DOE program is configured to be entirely self-sustaining, which gives us the growth potential and flexibility required to provide community-based education and technical support now and in the future. SMIG is a non-profit Minnesota corporation with expressed goals consistent with The Hormel Institute. It is governed by a board of directors selected from the community. The board ensures that our DOE program remains true to the community and its mission. This year, in a cooperative effort between SMIG and the Austin Utilities, Austin has a city-wide wireless system. Our efforts with many of the local school districts, non-public schools, small libraries, and non-profit organizations have improved network technology and Internet availability for public use. We are also helping area organizations with promotion and visibility by assisting with web development and web space.

Building Operations and Maintenance
Supervisor: Ronald Skjeveland

Serving as the building coordinator of the beautiful new facility has been great. We have set up laboratories for the new research section leaders and remodeled a space in the Annex basement for an X-ray diffraction system. As we settle into our new facility, we continue to increase the overall efficiency of its use.

Left to right, below: Norman Johnson, Randy Johnson, Ronald Skjeveland, Tom Wobschall, Duane Graff.

Building Operations and Maintenance

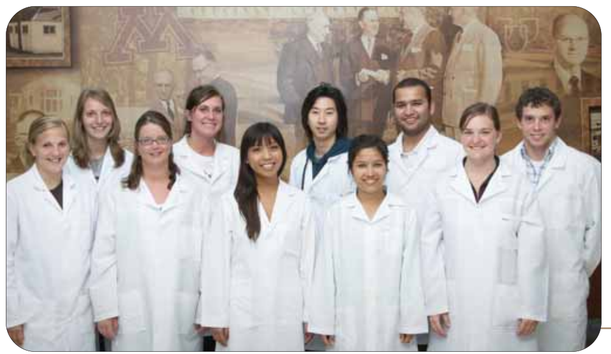
The maintenance support unit's main goal is to provide all personnel with a comfortable and safe working environment. Regular inspections and maintenance of all buildings and equipment is performed to assure continuous, efficient operation and comfort. All safety equipment is routinely checked to assure 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 rearrangment 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 tradesman 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 selected undergraduate students work in the Summer Undergraduate Research Experience (SURE) Program with The Hormel Institute scientists. The students work on research projects to expand their knowledge of basic research and to 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: Sarah Knopik, Gretchen Ramlo, Jay Wilson



Community Volunteers

The Hormel Institute receives important support from community volunteers each year for its many events for the public. In 2008-2009, volunteers assisted with tours and events for The Hormel Institute and included help from summer college interns, Jonathon Wilson and Sara Knopik. The Hormel Institute is thankful for its many volunteers who give of their time and talent so the Institute can share its cutting edge research and progress with visitors from the community and beyond.

Left to right, front row: Kelsey Kittleson, Tawnya Gardner, Diane Navoa, Joan, Twayna, Jennifer Welch, Timothy Finley

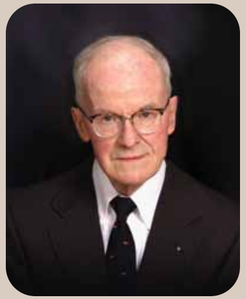
Back row: Leah Waters, Molly Pogones, Charles Changwon Lee, Paskal Pandy

Grand Opening October 2008

The expansion of The Hormel Institute made for a year of historic progress and exciting progress. Throughout 2006-2008 the project tripled the size of The Hormel Institute with the construction of a high impact, state-of-the-art research center. Twenty new labs are housed in the beautifully designed two-story building, along with a stunning lobby, several multi-purpose conference rooms and more.

"The result is a facility designed to support cutting-edge medical research and is the collaborative accomplishment of many. The expansion paves the way for our research to flourish and our progress to continue. We remain deeply grateful to our leaders and all who gave generously to make our vision become a reality."

Zigang Dong, M.D., Dr. P.H.
Executive Director, The Hormel Institute



"The expansion, renovation and increase in staffing of The Hormel Institute is a benefit to the Austin community now and for decades to come. Accomplishing it took vision, cooperation, funding and broad-based participation. Nothing but good will come from it."

I.J. Holton
Former Chair of The Hormel Foundation and former CEO, President and Chief Executive Officer of Hormel Foods Corporation

"Hormel Foods is excited about the opportunities this exceptional project is going to bring to our community. We made this donation because of the legacy and vision that began with Jay Hormel and the early investment of The Hormel Foundation. We are glad to be here to honor our brother entity because these are not ordinary jobs. These are scientists and researchers working on the prevention and control of cancer, a scourge that has touched us all."

Jeffrey M. Ettinger
President, CEO and Chairman of the Board
Hormel Foods Corporation

"Today we celebrate a new day for medical research with our new Hormel Institute. The expansion of this magnificent facility in partnership with the University of Minnesota and Mayo Clinic, and its research to attack cancer, is the most important accomplishment of The Hormel Foundation."

Richard L. Knowlton
Chairman,
The Hormel Foundation
Former President, CEO and Chairman of the Board
Hormel Foods Corporation
Officer of Hormel Foods Corporation

"The Hormel Institute is a successful collaboration of many groups – Hormel Foods, Mayo Clinic, the University of Minnesota, the City of Austin and also the federal support that comes from NIH – the National Institutes of Health. In Minnesota, we believe in science and technology. The accomplishments of The Hormel Institute are significant for our State and will help us grow in the important bioscience industry."

Senator Amy Klobuchar
U.S. Senate
Minnesota

"We are proud to be a partner supporting the important cancer research work of The Hormel Institute. Their collaborations with University of Minnesota and Mayo Clinic as well as research institutions around the world bring new and important jobs to rural Minnesota that compete with no others – and their work in cancer research is aimed for the public good. This is exactly the type of project that I will tirelessly advocate for in Congress because it makes sense both from a moral and a fiscal perspective."

Representative Tim Walz
U.S. House of Representatives
First Congressional District

"As Governor of this State, I am so proud of The Hormel Institute and the work that is done here. Its partnerships and research achievements support Minnesota's goal of leadership in bioscience and provides economic growth in an emerging industry in rural Minnesota. The Hormel Institute is doing exactly what Minnesota needs to in order to be competitive in today's world."

Governor Tim Pawlenty
State of Minnesota

"We are here to celebrate this new building but also our shared values, a shared vision for the future, a drive to discover, a hope for the future... I believe when we work together, in this great State of Minnesota, there's no problem in the world we can't solve and that includes the prevention and even the cure of cancer."

Robert H. Bruininks, Ph.D.
President
University of Minnesota

"Mayo Clinic is very proud of its historic partnership with The Hormel Institute going back nearly 7 decades... Mayo Clinic is committed to strengthening and building our partnership with The Hormel Institute and we are really excited about the things we will do in the future together. Our gift to the Institute expresses Mayo Clinic's dedication to our partnership."

Dr. Glenn S. Forbes
Chief Executive Officer
Mayo Clinic Rochester

World Cancer Conference

October 2008

The Hormel Institute Expansion Grand Opening including a three-day world cancer research conference held in Austin, Minnesota. Researchers from around the world gathered to share the most cutting edge information and technologies, furthering the progress of cancer prevention and control research.

"The Hormel Institute has established itself as a world-class research facility. With the fantastic new facility and infusion of additional faculty talent, the benefits to the University of Minnesota, the Austin region, the State of Minnesota and the nation will be spectacular."

R. Timothy Mulcahy, Ph.D.
Vice President for Research
University of Minnesota

"The progress of the The Hormel Institute through the expansion will take The Hormel Institute to greater heights of achievement in the years ahead, resulting in one of the leading R & D research facilities in the country."

Gary J. Ray
The Hormel Foundation and
Chairman
The Hormel Institute Expansion Project
Steering Committee

"The Hormel Institute's work in medical and agricultural applications reinforces many of the themes we found in Destination 2025, a 20-year strategic plan and roadmap to keep and expand Minnesota's competitive position in the biosciences. Their forward thinking is a reason why The Hormel Institute is a key leader and partner in Minnesota's bioscience and academic communities. We are grateful for their work and look forward to a deepening and expanding partnership."

Dale Wahlstrom
BioBusiness Alliance of Minnesota

"In my view, it is not far-fetched to dream of a day when our southern Minnesota region will be seen as a bio-agriculture, bio-medical, bio-energy "mecca" – not unlike the research triangle in North Carolina or Silicon Valley in California. The assets are here, planning is underway and collaborations are forming. The goal is within reach, and the world-recognized research of The Hormel Institute plays an important role in this vision."

Tim Penny
President & CEO
Southern Minnesota
Initiative Foundation

Hormel Institute Publications

July 1, 2008 — June 30, 2009

H.I. No. 1688
Resveratrol directly targets COX-2 to inhibit carcinogenesis
Zykova T, Zhu F, Zhai X, Ma WY, Ermakova SP, Lee KW, Bode AM, Dong Z
Mol Carcinog. Oct;47(10):797-805 (2008)

H.I. No. 1689
Mammary tumor development from T47-D human breast cancer cells in obese ovariectomized mice with and without estradoil supplements
Nkhata K, Ray A, Dogan S, Grande JP, Cleary MP
Breast Cancer Res. Treat. 114:71-83 (2009)

H.I. No. 1690
Intermittent calorie restriction delays prostate tumor detection and increases survival time in TRAMP mice
Bonorden M, Rogozina O, Kluczny C, Grossmann ME, Grambsch PL, Grande JP, Perkins S, Lokshin A, and Cleary MP
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Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite
Li GX, Lee HJ, Wang Z, Hu H, Liao JD, Watts JC, Combs GF Jr, Lü J
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Modulation of cell signal transduction by tea and ginger
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p21 activated kinase 5 activates Raf-1 and targets it to mitochondria
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(-)-Epigallocatechin gallate regulates CD3-mediated T cell receptor signaling in leukemia through the inhibition of ZAP-70 kinase
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PKR regulates B56alpha -mediated BCL2 phosphatase activity in acute lymphoblastic leukemia derived REH cells
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Quinoline derivative KB3-1 potentiates paclitaxel induced cytotoxicity and cycle arrest via multidrug resistance reversal in MES-SA/DX5 cancer cells
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Chemopreventive effect of kava on 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone plus benzo[a]pyrene-induced lung tumorigenesis in A/J mice
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The resveratrol analogue 3,5,3′,4′,5′-pentahydroxy-trans-stilbene inhibits cell transformation via MEK
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H.I. No. 1722
Epstein-Barr virus latent membrane protein 1 mediates serine 25 phosphorylation and nuclear entry of annexin A2 via PI-PLC- PKCalpha/PKCbeta pathway
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Transient receptor potential type vanilloid 1 suppresses skin carcinogenesis
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Signal transduction molecules as targets for cancer prevention
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H.I. No. 1729
Penta-O-galloyl-beta-D-glucose induces S-and G(1)-cell cycle arrests in prostate cancer cells targeting DNA replication and cyclin D1
Hu H, Zhang J, Lee HJ, Kim SH, Lü J
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Glycolipid acquisition by human glycolipid transfer protein dramatically alters intrinsic tryptophan fluorescence: insights into glycolipid binding affinity
Zhai X, Malakhova ML, Pike HM, Benson LM, Bergen HR 3rd, Sugár IP, Malinina L, Patel DJ, Brown RE
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H.I. No. 1731
In vivo molecular mediators of cancer growth suppression and apoptosis by selenium in mammary and prostate models: lack of involvement of gadd genes
Jiang W, Jiang C, Pei H, Wang L, Zhang J, Hu H, Lü J
Mol Cancer Ther. Mar;8(3):682-91 (2009)

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Cyclooxygenase-2/prostaglandin E2 pathway mediates icariside II induced apoptosis in human PC-3 prostate cancer cells
Lee KS, Lee HJ, Ahn KS, Kim SH, Nam D, Kim DK, Choi DY, Ahn KS, Lu J, Kim SH
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H.I. No. 1733
Minireview: Obesity and breast cancer: the estrogen connection
Cleary MP, Grossmann ME
Endocrinology. Jun;150(6):2537-42 (2009)

H.I. No. 1734
Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit
Wang L, Bonorden MJ, Li GX, Lee HJ, Hu H, Zhang Y, Liao JD, Cleary MP, Lü J
Cancer Prev Res (Phila Pa). May; 2(5):484-95 (2009)

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Methyl-selenium compounds inhibit prostate carcinogenesis in the transgenic adenocarcinoma of mouse prostate model with survival benefit
Wang L, Bonorden MJ, Li GX, Lee HJ, Hu H, Zhang Y, Liao JD, Cleary MP, Lü J
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A regulatory mechanism for RSK2 NH(2)-terminal kinase activity
Cho YY, Yao K, Pugliese A, Malakhova ML, Bode AM, Dong Z
Cancer Res. 69(10):4398-406 (2009)

H.I. No. 1737
Cyclin-dependent kinase 3-mediated activating transcription factor 1 phosphorylation enhances cell transformation
Zheng D, Cho YY, Lau AT, Zhang J, Ma WY, Bode AM, Dong Z
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H.I. No. 1738
Anti-androgen receptor signaling and prostate cancer inhibitory effects of sucrose- and benzophenone-compounds
Wang Z, Lee HJ, Wang L, Jiang C, Baek NI, Kim SH, Lü J
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H.I. No. 1739
Superior in vivo inhibitory efficacy of methylseleninic acid against human prostate cancer over selenomethionine or selenite
Li GX, Lee HJ, Wang Z, Hu H, Liao JD, Watts JC, Combs GF Jr, Lü J
Carcinogenesis. May;29(5):1005-12 (2009)

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Identification of the cyclin D1b mRNA variant in mouse
Wu J, Wu SH, Bollig A, Thakur A, Liao DJ
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H.I. No. 1741
Genome based cell population heterogeneity promotes tumorigenicity: the evolutionary mechanism of cancer
Ye CJ, Stevens JB, Liu G, Bremer SW, Jaiswal AS, Ye KJ, Lin MF, Lawrenson L, Lancaster WD, Kurkinen M, Liao JD, Gairola CG, Shekhar MP, Narayan S, Miller FR, Heng HH
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Effects of Adiponectin and leptin co-treatment on human breast cancer cell growth
Nkhata KJ, Ray A, Schuster TF, Grossmann ME, Cleary MP
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H.I. No. 1744
Y-box binding protein-1 serine 102 is a downstream target of p90 ribosomal S6 kinase in basal-like breast cancer cells
Stratford AL, Fry CJ, Desilets C, Davies AH, Cho YY, Li Y, Dong Z, Berquin IM, Roux PP, Dunn SE
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H.I. No. 1745
Cancer prevention research - then and now
Bode AM, Dong Z
Nature Reviews Cancer 9, 508-516 (2009)

H.I. No. 1746
Effects of gefitinib (Iressa) on mammary cancers: preventive studies with varied dosages, combinations with vorozole or targretin, and biomarker changes
Lubet RA, Szabo E, Christov K, Bode AM, Ericson ME, Steele VE, Juliana MM. Grubbs, CJ
Mol Cancer Ther, 7, 972-9 (2008)

H.I. No. 1748
Epigallocatechin 3-gallate and green tea catechins: United they work, divided they fail
Bode AM, Dong Z
Cancer Prev Res (Phila Pa), 2, 514-7 (2009)

Hormel Institute Seminars

July 1, 2008 — June 30, 2009

Rebecca Morris, Ph.D.
Columbia University, New York, NY
July 11, 2008
"Blue tumors and the origins of skin cancer in mice"

Maarten Bosland, Ph.D.
University of Illinois at Chicago
July 25, 2008
"Prostate cancer (chemo)prevention: studies with soy protein"

Mushui Dai, Ph.D.
Indiana University School of Medicine, Indianapolis, IN
August 11, 2008
"Regulation of p53 and c-Myc by ribosomal proteins and nucleostemin"

Wenlai Zhou, Ph.D.
University of California at San Diego
August 14, 2008
"Histone H2A ubiquitination in inflammation and cancer"

Edward Hinchcliffe, Ph.D.
University of Notre Dame, Indiana
August 19, 2008
"Centrosomes, spindles poles and cytokinesis – the end justified the means"

Yibin Deng, M.D., Ph.D.
M.D. Anderson Cancer Center
University of Texas, Houston, TX
August 21, 2008
"Mechanisms of tumor suppression: apoptosis, senescence and autophagy"

Allen Conney, Ph.D.
Rutgers, The State University of New Jersey, Piscataway, NJ
August 27, 2008
"Studies on cancer prevention: a path from tea to caffeine to exercise"

Xinjiang Wang, Ph.D.
Memorial Sloan-Kettering Cancer Center, New York, NY
August 28, 2008
"Identification of E3 ligases regulating tumor suppressor pathways"

Zhi-Xiang Xu, M.D., Ph.D.
M.D. Anderson Cancer Center
University of Texas, Houston, TX
September 2, 2008
"Avicin D, a plant triterpenoid, induces autophagy by activation of AMP-activated protein kinase"

Pat Ongusaha, Ph.D.
Harvard Medical School, Boston, MA
September 4, 2008
"Cellular stress & cancer: identification of key biological networks"

James McCubrey, Ph.D.
East Carolina University, Greenville, NC
September 12, 2008
"Targeting the PI3K/PTEN/Akt/GSK-3 pathway to eliminate breast cancer drug resistance"

Bhagavathi Narayanan, Ph.D.
New York University School of Medicine, New York, NY
September 25, 2008
"Molecular cross-talks mediated by anti-inflammatory agents in combination against prostate cancer"

Malathi Krishnamurthy, Ph.D.
Case Western Reserve University, Cleveland, OH
September 29, 2008
"Role of small self-RNA generated by RNase L in antiviral innate immunity and cancer"

Victor Fung, Ph.D.
Consultant (retired NIH Scientific Review Officer)
October 8, 2008
"Grantsmanship"

Prof. Lennart B.-Å. Johansson
Umeå University, Sweden
November 8, 2008
"Electronic energy transfer/migration & biomacromolecular structure"

Chung S. Yang, Ph.D.
Rutgers, The State University of New Jersey, Piscataway, NJ
December 1, 2008
"Tea and cancer prevention: molecular mechanisms and human relevance"

Yuxin Yin, Ph.D.
Columbia University, New York, NY
December 7, 2008
"PTEN is a powerful guardian of the genome"

Woo-Young Kim, Ph.D.
M.D. Anderson Cancer Center
University of Texas, Houston, TX
December, 30, 2008
"Dequelin, a novel anticancer therapeutic and chemopreventive agents blocking HSP90 function, overrides the radioresistance of lung cancer cells"

Gloria Petersen, Ph.D.
Mayo Clinic, Rochester, MN
January 30, 2009
"Mayo Clinic Cancer Center Pancreas SPORE grant: current activities and future opportunities"

David Largaespada, Ph.D.
University of Minnesota, Minneapolis, MN
February, 13, 2009
"Transposons as tools for oncology target discovery"

Brent Bauer, M.D.
Mayo Clinic, Rochester, MN
February 27, 2009
"The ambonese herbal: what a 400-year old text is teaching the Mayo Clinic"

Igor Roninson, Ph.D.
Ordway Research Institute, Albany, NY
March 25, 2009
"Transcriptional consequences of cellular damage: therapeutic implications for cancer and aging"

Robert Diasio, Ph.D.
Mayo Clinic Cancer Center,
Rochester, MN
March 27, 2009
"Fostering interactions between Hormel Institute and Mayo Clinic Cancer Center"

Shrikant Anant, Ph.D.
University of Oklahoma, Oklahoma City, OK
April 10, 2009
"RNA binding proteins as targets for cancer therapeutics: when all else fails, silence the messenger"

Stephen Ekker, Ph.D.
Mayo Clinic, Rochester, MN
April 24, 2009
"Bette Davis, Humphrey Bogart and the genetics of nicotine dependency"

Ann E. Hagerman, Ph.D.
Miami University, Oxford, OH
May 22, 2009
"Chemical and biochemical activities of plant polyphenolics"

Edward Leof, Ph.D.
Mayo Clinic Cancer Center,
Rochester, MN
May 29, 2009
"Regulation of TGF-beta receptor trafficking and cell type-specific signaling"

W. Stratford May, Jr., M.D, Ph.D.
University of Florida Shands Cancer Center, Gainesville, FL
June 5, 2009
"BCL2, apoptosis, p53 and all that JAZ(Z)"

Income from Grants and Contracts

National Institutes of Health
National Cancer Institute

Anticarcinogenic Mechanisms of Tea Constituents (Z. Dong)	*187,169*
Study on Ultraviolet-induced Signal Transduction (Z. Dong)	*91,954*
Intermittent Food Restriction Prevents Mammary Tumors (M. Cleary)	*116,627*
Selenium and Prostate Cancer Apoptosis Pathways (J. Lü)	
Chemoprevention of Skin Cancer Program Project (Z. Dong)	*193,212*
Mechanisms of Chemopreventive Effect of Resveratrol (Z. Dong)	*187,266*
Inhibition of Carcinogenesis by Tea and Tea Constituents (Z.Dong)	*172,353*
Molecular Basis of Glycosphingolipid Binding Specificity (R. Brown)	*71,000*
Measurement of Specific Signal Transduction Endpoints to Identify Potential Biomarkers (A. Bode)	
c-Myc, Growth Factor and Breast Cancer (D.J. Liao)	
Methyl Selenium for Prostate Cancer Chemoprevention (J. Lü)	*190,000*
Therapy of AML (P. Ruvolo)	*40,892*
2008 International Symposium (Dong)	*9,000*
Prostate Cancer Chemoprevention by Penta-galloyl-glucose (J. Lü)	*100,000*

National Heart, Lung, and Blood Institute

Regulation of Peripheral Protein-Membrane Interactions by Lipid Second Messengers (H. Brockman)	*259,509*

National Institute of Environmental Health Sciences

The Role of Histone Phosphorylation in Arsenic-induced Cell Transformation and Cancer (Z. Dong)	*225,000*

National Institute of General Medical Sciences

Glycolipid Transfer-Regulation by Membrane Interfaces (R. Brown)	*237,895*

Department of Defense – U.S. Army

Role of Obesity in Prostate Cancer Development (M. Cleary)	

American Institute for Cancer Research

Dietary Obesity and Prostate Cancer Development in TRAMP Mice (M. Cleary)	

Breast Cancer Research Foundation

Body Weight Change, Leptin/Adiponectin and Breast Cancer (M. Cleary)	

Kyunghee University (Seoul, Korea)

Identification of Novel Anti-androgen Compounds from Oriental Herbal Extracts (J. Lü)	*30,000*

Minnesota Medical Foundation

Pyranocoumarin Compounds for Breast Cancer Prevention and Treatment (J. Lü)	*20,000*

Minnesota Office of Higher Education

Virtual Screening for Designing Selective ERK Inhibitors (A. Bode)	

Minnesota Partnership for Biotechnology and Medical Genomics

Selective Small-molecule Inhibitors of JNK2 as Anti-cancer Drugs (Z. Dong)	

Pardee Foundation

Roles of the mir-17 and mir-221 Clusters of MicroRNAs in Breast Cancer (D.J. Liao)	

Pediatric Pharmaceuticals

Ginger as an Anti-cancer Agent (A. Bode)	
Full award amount stated in previous report	

Seoul National University (Korea)

Biogreen 21 Project (Z. Dong)	*37,525*

Susan G. Komen for the Cure

Calorie Restriction and Eicosapentaenoic Acid (M. Grossmann)	*100,831*

University of Minnesota Rochester

Virtual Screening for Designing Selective ERK Inhibitors (A. Bode)	*83,333*

Other Resources

The Hormel Foundation	*1,936,379*
University of Minnesota	*2,535,400*
Indirect Cost Return	*1,743,991*
Eagles Cancer Telethon	*130,000*
Mayo Clinic Collaborative Donation	*1,000,000*
Other	*250,090*
Total	**9,949,426**

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Ashok Singh, Hormel Fellow
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Craig Jones, Information Technology Supervisor
Michael Conway, Senior Electronics Technician
Theresa Tucker, Information Technology Professional
Timothy Lastine, Information Technology Professional
Rose Srock, Data Processing Technician
Ryan Wiersma, Data Processing Technician
Gary Bush, Technical Supplemental
Kokouvi Dokpodjo, Technical Supplemental
Darik Weitzel, Technical Supplemental

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