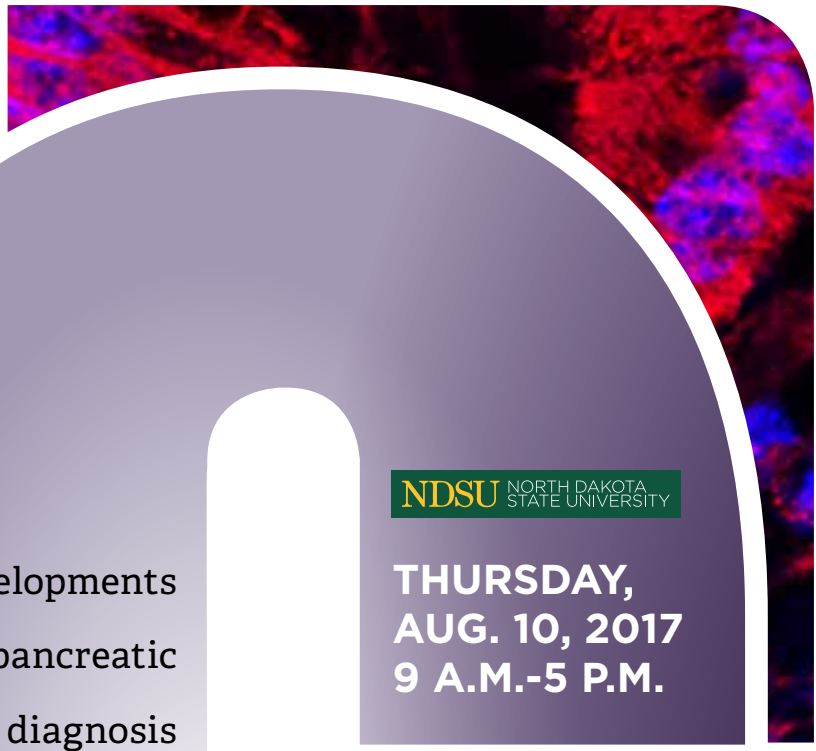


NDSU NORTH DAKOTA STATE UNIVERSITY

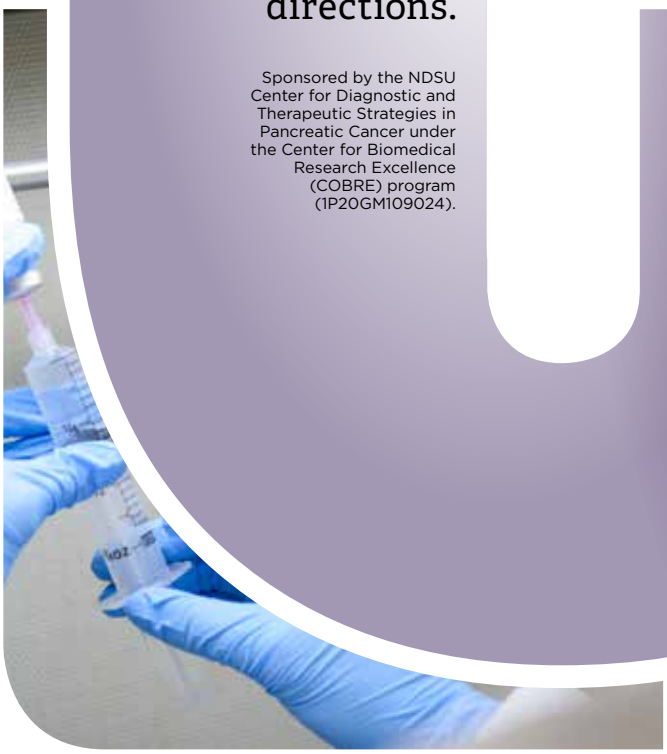
THURSDAY, AUG. 10, 2017
9 A.M.-5 P.M.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM

New developments in pancreatic cancer diagnosis and drug delivery. Learn, network and explore new research directions.

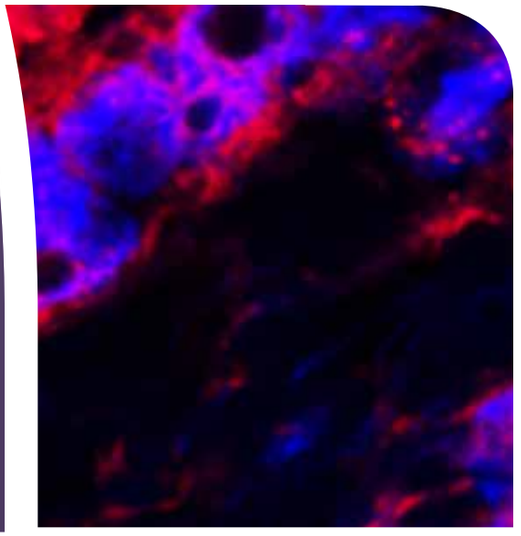
Sponsored by the NDSU Center for Diagnostic and Therapeutic Strategies in Pancreatic Cancer under the Center for Biomedical Research Excellence (COBRE) program (1P20GM109024).

Memorial Union
Great Plains Ballroom
North Dakota State University



WELCOME

www.ndsu.edu/centers/pancreaticcancer



Thank you for participating in the inaugural Pancreatic Cancer and Related Diseases Symposium at North Dakota State University.

It is an opportunity to learn about developments in pancreatic cancer diagnosis and drug delivery from exceptional leaders at the forefront of research, as we network and explore new research directions.

Sanku Mallik, Professor of Pharmaceutical Sciences, College of Health Professions
Director, NDSU Center for Diagnostic and Therapeutic Strategies in Pancreatic Cancer

D.K. Srivastava, James A. Meier Professor of Chemistry and Biochemistry, College of Science and Mathematics
Co-Director, NDSU Center for Diagnostic and Therapeutic Strategies in Pancreatic Cancer

SYMPOSIUM AGENDA

THURSDAY, AUGUST 10, 2017

8:30 a.m. Breakfast / Registration – NDSU Memorial Union, Plains Room
1401 Administration Avenue, Fargo

Session 1, Memorial Union, Great Room

9 a.m. Welcome and Opening Remarks
NDSU President Dean L. Bresciani
Dr. Kelly A. Rusch, Vice President for Research and Creative Activity, NDSU

9:30 a.m. "Stratifying Risk Groups for Early Detection of Pancreatic Cancer"
Gloria Petersen, Ph.D., Professor of Epidemiology, Mayo Clinic
Director - Pancreatic Cancer Specialized Programs of Research Excellence, National Institutes of Health

10:30 a.m. Break

10:45 a.m. "Advances in the Early Detection of Pancreatic Cancer"
Randall E. Brand, M.D., University of Pittsburgh Medical Center
Division of Gastroenterology, Hepatology and Nutrition
Director – GI Malignancy Early Detection, Diagnosis and Prevention Program of UMPC – Shadyside, Pittsburgh, Pennsylvania

11:45 a.m. Lunch / Poster Session – Memorial Union, Plains Room

Session 2, Memorial Union, Great Room

1:15 p.m. "Biomimetic and Bio-inspired Systems for Drug Delivery"
Samir Mitragotri, Ph.D., Harvard University
Hiller Professor of Bioengineering, John A. Paulson School of Engineering and Applied Sciences
Wyss Institute Professor of Biologically Inspired Engineering

2:15 p.m. "The Receptor for Advanced Glycation Endproducts (RAGE) Regulates Inflammation and Cancer: View from the T-cell"
Michael Lotze , M.D., University of Pittsburgh
Professor of Surgery and Bioengineering
Director of Strategic Partnerships, University of Pittsburgh Cancer Institute

3:15 p.m. Break

3:30 p.m. "Targeting KRAS in Pancreatic Cancer: Mission Possible?"
Adrienne Cox, Ph.D., University of North Carolina at Chapel Hill School of Medicine, Associate Professor in Radiation

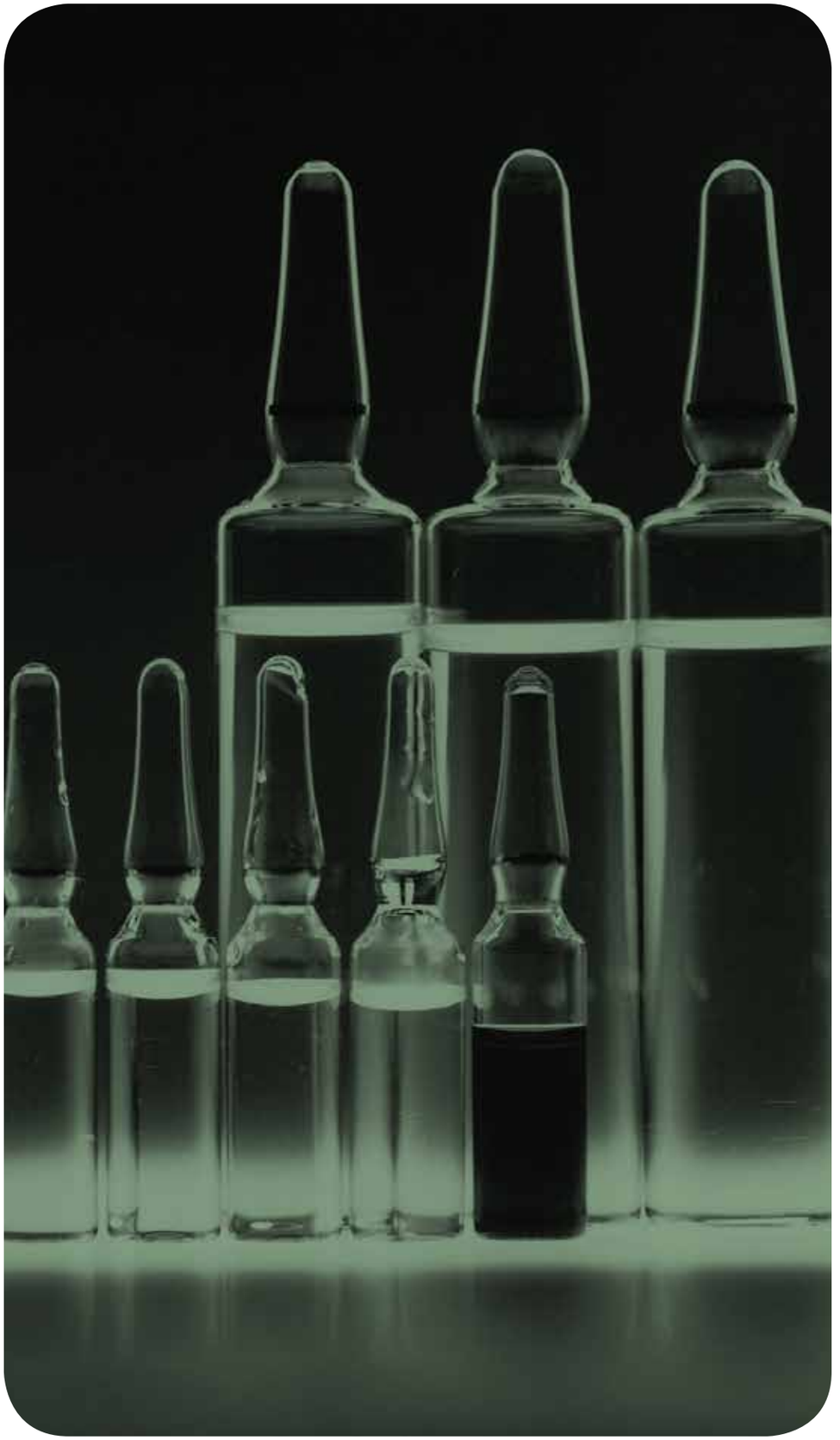
4:30 p.m. Closing Remarks
Dr. Scott A. Wood, Dean, NDSU College of Science and Mathematics
Dr. Charles D. Peterson, Dean, NDSU College of Health Professions

NDSU Harry D. McGovern Alumni Center – Reimers Room

5:30 p.m. Reception - Alumni Center, 1241 N. University Drive, Fargo

6:30 p.m. Dinner

7 p.m. Keynote Presentation
"New Strategies for Detecting and Treating Pancreatic Cancer"
Michael A. (Tony) Hollingsworth, Ph.D., University of Nebraska Medical Center
Dr. and Mrs. Tim D. Leon Professor, Eppley Institute for Research in Cancer
Associate Director for Basic Research, Fred and Pamela Buffett Cancer Center
Director - Pancreatic Cancer Specialized Programs of Research Excellence, National Institutes of Health



PRESENTER BIOGRAPHIES

Randall E. Brand, M.D.

Dr. Brand is a physician scientist with a long track record of research on the early detection of GI malignancies and GI hereditary cancers. He is the leader of the GI Hereditary Tumor Clinic at the University of Pittsburgh, which includes a high-risk pancreas cancer program. He is currently a professor of medicine, academic director of gastroenterology at Shadyside Hospital, and director of the GI Malignancy Early Detection, Diagnosis and Prevention Program at the University of Pittsburgh.

Dr. Brand possesses vast experience recruiting and collecting complete, accurate data and biospecimens from patients with pancreatic-cancer prone families, pancreatic cancer, pancreatic cysts and other gastrointestinal conditions. He is leader of the University of Pittsburgh's Pancreatic Adenocarcinoma Gene-Environment Registry (PAGER). The biospecimen repository developed as part of the PAGER study is nationally recognized and serves as an excellent resource for multiple funded projects. He is a key contributor to the Early Detection Research Network. He has two U01 projects funded through the network as lead principal investigator of a pancreatic cancer clinical validation center and co-principal investigator of a biomarker developmental laboratory.

He has held faculty positions at the University of Nebraska as an assistant professor of medicine; in gastroenterology at Evanston Northwestern Healthcare; and associate professor of medicine at Northwestern University, Feinberg School of Medicine. Dr. Brand completed his medical degree and internal medicine residency at the University of Michigan, Ann Arbor, and a gastroenterology fellowship at the University of California, San Francisco.

Adrienne D. Cox, Ph.D.

Dr. Cox is chief of the Division of Cancer Research in the Department of Radiation Oncology at the University of North Carolina at Chapel Hill (UNC-CH), associate professor of pharmacology, and a member of the Lineberger Comprehensive Cancer Center (LCCC).

She has been involved in the study of RAS proteins since the discovery of their lipid modification by prenylation, publishing more than 100 articles in peer-reviewed journals and consulting for numerous pharmaceutical companies. Dr. Cox's research encompasses basic science aspects of RAS family signaling and transformation, as well as translational aspects such as the development of farnesyltransferase inhibitors, originally intended to be anti-RAS therapeutics. Her current studies focus on understanding and inhibiting the context-dependent roles of wild type RAS and distinct RAS mutations in melanoma, pancreatic and colon cancers.

Dr. Cox earned her B.A. from Pomona College, Claremont, California, her Ph.D. from Eastern Virginia Medical School, Norfolk, Virginia, and performed her postdoctoral studies at the La Jolla Cancer Research Foundation (now the Sanford Burnham Prebys Medical Discovery Institute), La Jolla, California.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

Michael A. (Tony) Hollingsworth, Ph.D.

The general subject of Dr. Hollingsworth's research is pancreatic cancer and other diseases of the pancreas, primarily pancreatitis. The major focus of the basic science studies is the role of MUC1 and other cell surface and extracellular proteins in metastasis and progression of pancreatic cancer.

Dr. Hollingsworth has served on numerous review panels at the National Institutes of Health and for other granting agencies, including recent service as Chair of the Cancer Biomarkers Study Section (CBSS), and was appointed by NIH Director Dr. Francis Collins in 2013 to serve as a member of the Center for Scientific Review Advisory Council. He participates as a member of the Scientific and Medical Advisory Board for PanCAN (Pancreatic Cancer Action Network), and has participated in almost every major initiative from NIH related to pancreatic cancer since 1985.

He has a publication record of over 200 publications and 15 book chapters. Dr. Hollingsworth was honored as a UNMC Distinguished Scientist in 2006 and 2010, and named the UNMC Scientist Laureate in 2012. He received his Ph.D. from the Bowman Gray School of Medicine of Wake Forest University, Winston-Salem, North Carolina. Since 1985, he has committed his research career to pancreatic cancer, working on translational research projects in that area and maintaining R01 and other National Institutes of Health funding as principal investigator for projects related to pancreatic cancer. He moved to the Eppley Institute in 1991 where, in recent years, he has led the group effort in pancreatic cancer research, establishing a program of research that is funded, in part, by the National Cancer Institute. Dr. Hollingsworth has served as the principal investigator of a Specialized Program of Research Excellence (SPORE) in Pancreatic Cancer since 2008.

Michael T. Lotze, M.D.

Dr. Lotze is a clinician scientist who has spent the last decade assembling a team to work on the extraordinary problem of pancreatic cancer. He is professor of surgery, immunology, and bioengineering; vice chair of research within the Department of Surgery; assistant vice chancellor in the six schools of the Health Sciences; and co-director of cytometry within the University of Pittsburgh Cancer Institute, as well as director of the UPCI International Academy.

His research includes modern immunotherapy and gene therapy, dendritic cell and cytokine therapies, and investigation of the role of mitochondria, metabolism, and unscheduled cell death in cancer. With Herbert J. Zeh, M.D., a premier pancreatic surgeon and scientist and Daolin Tang, M.D., Ph.D., a creative and energetic molecular biologist, he created the Center for Damage Associated Molecular Pattern Molecule (DAMP) Biology within the University of Pittsburgh Cancer Institute. He also is an award-winning NCI-trained scientist, the inaugural director of Surgical Oncology at Pitt, former vice president of research at GlaxoSmithKline, and innovative educator who developed the UPCI International Academy, and a prolific scientist/tumor immunologist with over 500 publications and several books.

Dr. Lotze was appointed chief scientific officer and vice president of research and development, of Lion Biotechnologies, Inc., a company that is developing novel cancer immunotherapies based on tumor-infiltrating lymphocytes (TIL) on April 1, 2016. He is a renowned expert in immuno-oncology with more than 40 years of clinical experience. Having initiated the first approved gene therapy protocols at the National Institutes of Health, Dr. Lotze has treated over 100 patients on gene therapy at the University of Pittsburgh. He is the co-inventor of 10 patents in dendritic cell vaccines and antigen discovery. He also serves as associate editor of the Journal of Immunotherapy, Cancer Gene Therapy, Immuno-oncology, and Nature Regenerative Medicine.

Samir Mitragotri, Ph.D.

Dr. Mitragotri is a Hiller Professor of Bioengineering and Wyss Professor of Biologically Inspired Engineering at Harvard University. He previously served as the Mellichamp Chair Professor in the Department of Chemical Engineering at the University of California, Santa Barbara.

His research focuses on transdermal, oral, and targeted drug delivery systems. Dr. Mitragotri is an elected member of the National Academy of Engineering, National Academy of Medicine and National Academy of Inventors. He is also an elected fellow of American Association for the Advancement of Science, CRS, Biomedical Engineering Society, American Institute for Medical and Biological Engineering, and the American Association of Pharmaceutical Sciences.

Dr. Mitragotri has written more than 210 publications, been an inventor on more than 150 patent/patent applications, and a 2015, 2016 Thomson Reuters Highly Cited Researcher. He received his bachelor's degree in chemical engineering from the Institute of Chemical Technology, India and a Ph.D. in chemical engineering from the Massachusetts Institute of Technology. He serves as editor-in-chief of the American Institute of Chemical Engineers and the Society for Biological Engineering's new journal, Bioengineering and Translational Medicine.

Gloria M. Petersen, Ph.D.

Dr. Petersen serves as professor of epidemiology and holds the Purvis and Roberta Tabor Professorship at Mayo Clinic. She is deputy director for Population Sciences in the Mayo Clinic Cancer Center and a founding fellow of the American College of Medical Genetics and Genomics.

She studies the genetic epidemiology of pancreatic cancer, improving risk strategification, and early detection. Dr. Petersen is the Contact MPI of the Mayo Clinic Specialized Program of Research Excellence (SPORE) in Pancreatic Cancer, and the bioethics-focused Genomic Incidental Findings Disclosure. She leads a major research effort on early detection of pancreatic cancer at Mayo Clinic and is a key member of the leadership teams of the PanScan, PACGENE, and PanC4 consortia.

Before joining Mayo Clinic with joint appointments in Medicine and Medical Genetics, Dr. Petersen had faculty appointments at UCLA School of Medicine and Johns Hopkins University School of Public Health. She is co-principal investigator of the Mayo Cancer Genetic Epidemiology Training Program and serves on the Board of Scientific Counselors for the Human Genome Research Institute and the National Cancer Institute's Clinical and Translational Research Advisory Committee. Dr. Petersen received her Ph.D. in physical anthropology from the University of California, Los Angeles, her master's degree in physical anthropology from the University of Oregon, Eugene and a bachelor's degree in the same discipline from the University of California, Santa Barbara.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

POSTER PRESENTATIONS / ABSTRACTS

Direct Bio-printing with Heterogeneous Topology Design

AMM Nazmul Ahsan, Ruinan Xie, and Bashir Khoda
Department of Industrial and Manufacturing Engineering, College of Engineering, North Dakota State University, Fargo, ND

This research presents a topology based tissue scaffold design methodology to accurately represent the heterogeneous internal architecture of tissues/organs. In bio-fabrication processes, the generated bio-models with boundary representation (B-rep) or surface tessellation (mesh) do not capture the internal architectural information. This research provides a design methodology for scaffold structure mimicking the native tissue/organ architecture and direct fabricating the structure without reconstructing the CAD model.

An image analysis technique is used that digitizes the topology information contained in medical images of tissues/organs. A weighted topology reconstruction algorithm is implemented to represent the heterogeneity with parametric functions. The parametric functions are then used to map the spatial material distribution following voxelization. The generated information is directly transferred to the 3D bio-printer and heterogeneous porous tissue scaffold structure is manufactured without STL file. The proposed methodology is implemented to verify the effectiveness of the approach and the designed example structures are bio-fabricated with a deposition based bio-additive manufacturing system. The designed and fabricated heterogeneous structures show conforming porosity distribution compared to the uniform porosity scaffold structures. Therefore, designing and direct bio-printing the heterogeneous topology of tissue scaffolds from medical images minimize the disparity between the internal architecture of target tissue and its scaffold.

Gold Nanoparticle-based Lateral Flow Strip Biosensor for Rapid and Sensitive Quantitation of CA 19-9 in Human Plasma

Kwaku Baryeh, Sunitha Takalkar, Michelle Lund, Guodong Liu
Department of Chemistry and Biochemistry, College of Science and Mathematics, North Dakota State University, Fargo, ND

We present gold nanoparticle (GNP) -based lateral flow strip biosensor (LFSB) for rapid and sensitive detection of Human CA 19-9 antigen in human plasma. CA 19-9 is a biomarker that has been associated with cancers (such as pancreatic and colorectal cancers) and various non-cancerous diseases. The assay was based on the capture of target CA 19-9 antigen in a sandwich-type assay between an immobilized anti-CA 19-9 antibody and GNP-labeled detection antibody. The accumulation of GNPs on the test zone of LFSB gave a red colored line whose intensity was read with a portable strip reader to quantify the concentration of CA 19-9.

Assay parameters including the membrane type, antibody concentration, amount of anti-CA 19-9-GNP conjugates used as well as the components of the running buffer were optimized to give the best sensitivity and reproducibility. The detection limit of the assay was determined to be 5 U mL^{-1} (S/N=3) with a linear dynamic range of 5 U mL^{-1} to 100 U mL^{-1}. CA 19-9 concentrations in human plasma samples were successfully evaluated using the developed assay and the outcome was in accordance with enzyme linked immunosorbent assay (ELISA) results. This shows the potential of the developed assay for the rapid, low cost and sensitive detection of CA 19-9 in clinical samples.

Peptide Conjugated Hypoxia Sensitive Polymersomes for Targeted Pancreatic Cancer Delivery

Matthew Confeld
Department of Pharmaceutical Sciences, College of Health Professions,
North Dakota State University, Fargo, ND

Preventing unnecessary patient harm is an obstacle when using chemotherapeutic agents for cancer treatment. The medications are often given systemically and have no differentiation between what are normal cells and those that are cancerous. To improve patient outcomes, medications must be altered in such a way that they have a preferred target. This study uses a pancreatic cancer targeting peptide combined with a hypoxia sensitive polymer to prevent unwanted toxicity to the normal cell populations, with a simultaneous increase in effectiveness of treatment.

Polymersomes are nanovesicles composed of repeating molecular units, polymers, that can form spherical structures, containing a hydrophobic core and a hydrophilic outer layer. Utilizing a hypoxia sensitive linker, the polymersome can penetrate deep into the cancerous tissue and instill cell death from the inside. Here we used two chemotherapeutic agents, Gemcitabine and Napabucasin. Gemcitabine, a nucleotide analog, is incorporated into DNA and prevents further replication and Napabucasin, a stem cell inhibitor, are encapsulated into polymersome nanovesicles containing a 4,4' azobenzene linker. A small peptide known as iRGD that is capable of binding av integrins receptors overexpressed on cancerous cells is conjugated and incorporated into the polymersome structure. Utilizing both 2-D and 3-D cellular architectures, we can show the increased efficacy of using targeted, environment sensitive polymersomes over standard free drug formulations.

Regulation of Cell Fate Decisions Through the AIF/PGAM5 Axis

Kaitlin M. Dailey, Audrey M. Lenhausen, Amanda S. Wilkinson, and John C. Wilkinson
Cell and Molecular Biology Program, Department of Chemistry and Biochemistry,
College of Science and Mathematics, North Dakota State University, Fargo, ND

AIF is a mitochondrial flavoprotein that plays a role in cell death and survival. The balance between AIF activities is not fully understood. PGAM5 binds AIF and has been indicated in pathways including antioxidant response and forms of cell death, suggesting that PGAM5 may participate in AIF signaling. Two isoforms have been identified, $PGAM5_S$ and $PGAM5_L$, sharing a common amino-terminus but differing at the carboxy-terminus. $PGAM5_L$ is a protein phosphatase, an activity also ascribed to $PGAM5_S$ based on sequence similarity. We explore the influence that PGAM5 may exert over cell fate decisions such as apoptosis both with and without AIF.

Piperlongumine Potentiates the Effects of Gemcitabine in In Vitro and In Vivo Human Pancreatic Cancer Models

Mohammad J[a], Dhillon H[a], Chikara S[a], Mamidi S[b], Sreedasyam A[b], Chittem K[c], Orr M[d], Wilkinson J[e], Reindl KM[a]
[a]Department of Biological Sciences, College of Science and Mathematics, North Dakota State University, Fargo, ND
[b]HudsonAlpha Institute for Biotechnology, Huntsville, AL
[c]Department of Plant Pathology, North Dakota State University, Fargo, ND
[d]Department of Statistics, North Dakota State University, Fargo, ND
[e]Department of Chemistry and Biochemistry, North Dakota State University, Fargo, ND

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

Pancreatic cancer is one of the deadliest cancers due to a late diagnosis and poor response to available treatments. There is a need to identify complementary treatment strategies that will enhance the efficacy and reduce the toxicity of currently used therapeutic approaches. Cancer cells have high basal ROS levels which, when elevated further, trigger cell death. Therefore, reactive oxygen species (ROS)-inducing agents hold potential as selective anti-cancer agents.

We investigated the ability of a known ROS inducer, piperlongumine (PL), to complement the anti-cancer effects of the chemotherapeutic agent gemcitabine (GEM) in pancreatic cancer cells in vitro and in vivo. Pancreatic cancer cells (MIA PaCa-2 and PANC-1) treated with GEM + PL showed significant elevation of ROS levels and reduction in cell proliferation, cell viability, clonogenic survival, and migration compared to control and individually-treated cells. Nude mice bearing orthotopically implanted MIA PaCa-2 cells treated with PL (5 mg/kg) and/or GEM (25 mg/kg) had significantly lower tumor weight and volume compared to control and single agent-treated mice.

RNA sequencing (RNA-Seq) revealed the combination therapy (GEM + PL) resulted in significant gene expression changes related to cell death, cellular stress responses, cell cycle, and DNA repair pathways. We propose a mechanism for the complementary anti-tumor effects of GEM and PL in pancreatic cancer cells through elevation of ROS leading to cell cycle arrest and induction of apoptosis. Collectively, our results suggest that PL has a potential to be used in combination with gemcitabine to more effectively treat pancreatic cancer.

Synthesis of Hypoxia-responsive Block Copolymers

Li Feng, Matthew Confeld, Fataneh Karandish, Sanku Mallik
Department of Pharmaceutical Sciences, College of Health Professions,
North Dakota State University, Fargo, ND

Hypoxia in solid tumors, tumor hypoxia, is a classical, unique feature of cancer caused by the rapid cell division and inadequate supply of oxygen due to their rapid growth. Hypoxia promotes epithelial to mesenchymal transition and shelters the cancer stem cells. We are interested in preparing hypoxia-responsive polymeric drug carriers (polymersomes) to deliver anticancer drugs and kill the malignant as well as the cancer stem cells. In order to eliminate tumor, the most efficient and safe way is to kill only the differentiated cancer cells in the hypoxia microenvironment of tumor, avoiding the side-effects to healthy tissues. Hypoxia-responsive conjugated copolymers have been developed for drug delivery to hypoxic cancer cells. Compared to the conventional chemotherapy, polymersomes with anticancer drug encapsulated show reduced side effects, with the drugs released specially in tumor.

In this study, we have synthesized a series of hypoxia-responsive, diblock copolymers poly(lactic acid)–azobenzene–poly(ethylene glycol) with different molecular weights and slight changes of structurehypoxia-sensitive linkers. The polymers, which self-assemble to form polymersomes in an aqueous medium, were prepared by the solvent exchange method.

Under reducing hypoxic conditions, the azobenzene linker undergoes reduction, the polymer bilayer collapses, releasing the encapsulated anticancer drugs. The released drugs decreased the viability of cultured pancreatic cancer cell spheroids. We observed that the polymeric vesicles are stable under normoxia and do not release the drugs and the encapsulated drugs will be released from the polymersomes into the pancreatic cancer cells, while the drugs could not be released in normoxic cells to avoid their toxicity to healthy tissues. We demonstrated the efficient release of encapsulated anticancer drugs from the polymersomes under hypoxic conditions in pancreatic cancer cells. The drug—encapsulated, hypoxia-responsive polymersomes—a might open up a new route for cancer therapy.

Hydrogel Based Scaffold Characterization for Bio-printing

Md Ahasan Habib and Bashir Khoda
Department of Industrial and Manufacturing Engineering, College of Engineering,
North Dakota State University, Fargo, ND

Due to the inadequacy of animal models to predict the applicability of drug and other physiological behavior, the in-vitro models become a vital mean that can recreate the in vivo scenario. Bio-printing is a revolutionary technology uses a computer-controlled 3D printing discipline to reproduce a 3D functional living tissue scaffold in-vitro through controlled layer-by-layer deposition of biomaterials along with high precision positioning of cells. Several bio-printing modalities are currently being explored (i.e. laser, extrusion, inkjet, and soft lithography) in literature for tissue engineering applications.

Hydrogels play an imperative role in 3D-bioprinting to encapsulate, mature and proliferate the cell and eventually form extra-cellular matrix (ECM). In this poster, the characterization of the hydrogels for extrusion-based bio-printing will be studied to find the appropriate combination of hydrogels and process parameters for cell survivability and proliferation. The future work of this research will investigate the behavior of scaffold architecture on cell behaviors (migration and proliferation), suitable fabrication parameters and develop a perfusion based bio-reactor. The successful implementation of this research will nourish the regeneration of heterogenous/vascularized functional tissue and a great impact in tissue engineering and regenerative medicine in the long run.

COBRE Center for Diagnostic and Therapeutic Strategies in Pancreatic Cancer Core Research Facility

Director: Dr. Steven Qian / Manager: Dr. Jodie Haring
North Dakota State University, Fargo, ND

The facility provides suitable space, support, and training for cancer research involving small animals campus-wide. We support both subcutaneous and orthotopic tumor models, and are developing standard operating procedures for patient derived xenografts (PDX). The Core Research Facility and operating procedures can accommodate projects requiring ABSL-1 and ABSL-2 containment, and models requiring the use of immunocompromised rodents. We provide assistance beginning with the development of your protocol, provide training for investigators, and support experimental and data collection needs during the project. If you have interest in learning more about our capabilities, contact facility personnel to set up an appointment to discuss your project, fee structure, and additional information.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

Peptide-targeted, Stimuli-responsive Polymersomes for Delivering a Cancer Stemness Inhibitor to Prostate Cancer Stem Cells

Fataneh Karandish, John Wilkinson, and Sanku Mallik
Department of Pharmaceutical Sciences, College of Health Professions,
North Dakota State University, Fargo, ND

Chemotherapy is the most common treatment for cancer. Often cancer relapses after initial response to the chemotherapy. Tumor cells are heterogeneous and have the progenitor stem cells, which can renew, causing the relapse of the disease. To overcome drug resistance, metastasis, and relapse in cancer, a promising approach is the inhibition of cancer stemness. We are designing a targeted stimuli-responsive, polymeric nanocarrier to deliver a stemness inhibitor (BBI608) to the cancer stem cells.

We synthesized a reduction-sensitive amphiphilic block copolymer PEG-S-S-PLA and the N_3-PEG-PLA. The iRGD-hexynoic acid-peptide conjugate was prepared by employing a microwave assisted, solid phase peptide synthesizer iRGD-hexynoic acid was conjugated to the N_3-PEG-PLA polymer employing the Cu^{2+} catalyzed “Click” reaction to incorporate the targeting group in the polymeric vesicles (polymersomes). The product of the reaction was characterized by FT-IR spectroscopy. We prepared polymersomes containing 85 percent PEG-S-S-PLA, 10 percent iRGD-HEX-PLA-PEG and 5 percent lissamine rhodamine dye. Control and iRGD targeted polymersomes encapsulating BBI608 were prepared by the solvent exchange method. The drug encapsulated and the control polymersomes had an average size of 220 3 20 nm, with polydispersity index of 0.2 3 0.01. BBI608 release from targeted polymersomes was performed in the presence of glutathione (GSH).

We observed that 55 percent of encapsulated drug was released with 5 mM GSH. The high amount of reducing agent in the cancer stem cells released the encapsulated cancer stemness inhibitor from the polymersomes. The BBI608 encapsulated polymersomes significantly ($p < 0.05$) decreased the viability of the prostate cancer stem cells compared to the control and polymersomes without any drug. Cell apoptosis was determined by flow cytometry. The BBI608 encapsulated polymersome formulations have the potential to lead to a new direction in prostate cancer therapy by killing the cancer stem cells.
Acknowledgment: Dr. Manas Haldar for synthesis polymers, NIH 1R01 GM114080 for funding.

Piperlongumine Activates JNK Signaling in Pancreatic Cancer Cells

Jagadish Loganathan, Rahul Raj Singh, Katie M. Reindl
Department of Biological Sciences, College of Science and Mathematics,
North Dakota State University, Fargo, ND

Pancreatic ductal adenocarcinoma has an extremely high mortality rate, warranting investigation of new therapeutic interventions to help increase survival in patients. Piperlongumine (PL), a plant-derived natural compound, selectively kills cancer cells through elevation of oxidative stress by binding to and inhibiting glutathione S-transferase pi 1 (GSTP1). GSTP1 is overexpressed in many cancers, including pancreatic cancer, where it detoxifies electrophilic compounds and suppresses JNK signaling. In the present study, we investigated the anti-proliferative effects of PL on two different pancreatic cancer cell lines (PANC-1 and MIA PaCa-2) using an MTT assay. Further we determined the effects of PL on JNK signaling using western blotting and the GSTP1-JNK interaction using co-immunoprecipitation.

The results show that PL decreased cell proliferation in time and concentration-dependent manners with an IC_{50} value of 1.98 QM and 3.3 QM for MIA PaCa-2 and PANC-1 cells at 72 hr, respectively. In addition, PL at 10 QM resulted in JNK phosphorylation within 15 minutes and up to 1 hr, after which phospho-JNK levels declined. Similarly, PL elevated c-Jun phosphorylation, a signaling protein downstream of JNK. Phosphorylation of c-Jun by PL was inhibited by the JNK inhibitor SP600125. Growth studies are on-going to determine if the JNK inhibitor blocks PL-induced pancreatic cancer cell death. Finally, PL resulted in a slight decrease in GSTP1 expression in a JNK pull-down assay, suggesting that PL may disrupt the association between GSTP1-JNK, and activate the JNK/c-Jun/AP-1 pathway leading to cell death. Knowledge of the mechanisms for PL-induced pancreatic cancer cell death may pave the way for combining PL with other agents currently used to treat this disease.

RAGE and its S100 Protein Ligands in Human Pancreatic Cancer Cells

Angelo Mandarino
Department of Pharmaceutical Sciences, College of Health Professions, North Dakota State University, Fargo, ND

Pancreatic cancer represents one of the most important causes of cancer death all over the world and is responsible for about 6 percent of all cancer deaths. In 2020, it is predicted to be the second cause of cancer death in the United States. Diagnosis of pancreatic cancers usually occurs when it is already too late for patients, and less than 6 percent of patients survive longer than five years. The most common pancreatic cancer is the pancreatic ductal adenocarcinoma (PDAC). The first event in PDAC is usually the formation of pancreatic intraepithelial neoplasia, PanIN-1, which progress to PanIN-2 and PanIN-3 leading to PDAC.

DiNorcia and coworkers recently showed that the Receptor for Advanced Glycation End products (RAGE) could play a key role in advancement of pancreatic cancer, and could therefore be a potential therapeutic target: the authors showed that deletion of RAGE in mice was delaying PanIN development and progression to PDAC.

RAGE can be activated by structurally unrelated ligands. An important group of ligands is formed by the S100 protein family. Many S100 members including S100A2, S100A4, S100A6, S100A8, S100A10, S100A11 and S100P have been shown to participate to pancreatic cancer growth. To better understand the role of RAGE and its S100 protein ligands in pancreatic cancer, we determined here the S100 protein signature of selected human pancreatic cancer cell lines (AsPC-1, BxPC-3, Capan-2, CFPAC-1, HPAF-II, Hs 766T, MIA PaCa-2, and PANC-1) both at transcript and protein levels. The levels of S100 proteins were correlated with the levels of RAGE in these cells.

Targeted Calcification of Cancer Cells Through the Use of Polymersomes: The More Effective, Less Toxic Method

Hope Osborn
North Dakota Governor's Schools

The use of polymersomes as drug carriers has proven itself useful when attempting to treat cancer stem cells. In this experiment, polymersomes encapsulating a high concentration of calcium chloride were used. The ability and efficiency of this treatment was tested on prostate stem cancer cells. When the stem cells are treated with the polymersomes (with or without the targeting iRGD peptide) encapsulating calcium chloride, the amount of cancer cell death increases. This proves that this approach is effective and can continue to be studied for possible use in the treatment of prostate cancer.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

Drug Loaded pH Responsive Polymeric Nanoparticles as Efficient Drug Delivery Agents for Pancreatic Cancer

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Pancreatic cancer is believed to be one of the most virulent forms of all cancers owing to its difficult diagnosis and limited therapeutic options. The pancreatic cancer microenvironment comprises an assortment of extracellular matrix and nonneoplastic cells including fibroblastic, vascular, and immune cells. Research has revealed that the pancreatic ductal adenocarcinoma (PDA) stroma supports tumour growth and promotes metastasis. Hence, it is important not only to combat the affected cells but also the cells on which these cells thrive. In order to stop the metabolic cross talk between stromal cells and cancer cells, we are in the process of developing a series of pH responsive polycarbonate polymers. As the microenvironment in the cancerous tissues and cellular compartments have large differences in pH, we have chosen pH as a suitable stimulus for smart drug delivery systems. It is known that the pH changes from an acidic range (~2) in the stomach to a basic one in the intestine (ranging from 5-8). Many carcinoma cells would also be expected to exhibit a pH different from 7.4 and extracellular part of cancerous tissues have been reported to have an acidic pH. The drop in pH is being utilised in this project to release the drugs selectively in the targeted zone.

The polymers we have synthesised consist of a hydrophobic and a hydrophilic block, which are pH responsive and form nanoparticles when using selective biocompatible solvents. These polymers form self-assemblies capable of encapsulating drugs and releasing them at acidic pH. The critical aggregation concentrations of both polymersomes were found to be of the order of 10^{-5} and 10^{-6} for the dibutyl amine and pyrrolidine amine respectively. The dibutyl amine polymersomes were found to have a loading efficiency of 89 percent for Gemcitabine HCl and showed a release of 96 percent at the end of 24 hours at pH 4.5 and a mere 52 percent at pH 5.5. This differential release will help the polymersomes in delivering the frontline chemotherapy drug right at the site of action whereby DNA replication and repair will be hindered. For the hydrophobic Hedgehog inhibitor (Vismodegib/ GDC 0449) our pyrrolidine polymersomes had a 26 percent loading efficiency and ~ 62 percent of the drug released at pH 5.5 (extracellular pH).

Both drugs showed a minimal release at physiological pH (7.4) thereby indicating the reduction of toxicity of the drugs to healthy cells and minimal loss of drugs in the blood. One of the polymersomes also will be labelled with a tumour penetrating peptide for a more specific drug delivery system. Cytotoxicity and in vivo studies will provide us with a complete picture of the intake, circulation, retention and the expulsion of the prepared nanosystems in living organisms.

Mitochondrial Oxidoreductase AIF Functions as a Novel Regulator of Cell Redox Signaling

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Apoptosis-inducing factor (AIF) is a mitochondrial oxidoreductase critical for maintaining cellular homeostasis. Distinct from its roles in cell death, AIF possesses an NADH-oxidase activity that generates superoxide but whose redox control mechanisms are not well understood.

Using a panel of human cell lines, we observed a spectrum of patterns for AIF-dependent respiratory chain control that partially required the enzymatic activity of AIF, and the ability of AIF to regulate reactive oxygen species (ROS) and the antioxidant response is dissociable from its metabolic activities. While AIF is not required for basal mitochondrial activity or abundance, we identified a novel role for AIF as an amplifier of both mitochondrial and cytosolic ROS that activate the mitogen-activated protein kinases c-Jun N-terminal kinase 1 (JNK1) and p38. AIF-dependent JNK1 signaling culminates in the cadherin switch, which is lethal in the absence of AIF. Thus the NADH-oxidase activity of AIF is a driver of pro-oxidant signaling pathways, and through its enzymatic activity AIF is a major mitochondrial sensor and effector of both cellular redox and metabolic state with important implications for human disease.

GSTP1 Knockdown and Inhibition Impairs Pancreatic Ductal Adenocarcinoma Growth (PDAC)

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Cancer is a complex group of diseases that results from uncontrolled cell division. Incessant cell proliferation is metabolically taxing, and requires a perpetual supply of energy, reducing power, and cellular building blocks such as amino acids, fatty acids, and nucleotides for protein, lipid, and nucleic acid synthesis, respectively. Tumor metabolism has recently risen to prominence when oncologists started studying how cancer cells obtain energy and cellular constituents for sustained growth. Apart from glucose and glutamine, inhospitable microenvironments of certain cancers, like pancreatic cancers, drive the need to scavenge alternative substrates, particularly proteins and lipids.

Through comprehensive metabolite profiling, three discrete subtypes of pancreatic ductal adenocarcinoma (PDAC) have been identified: one with reduced proliferative capacity, and the other two with glycolytic and lipogenic phenotypes. Glycolytic cancer cells can fulfill their demand for energy and building blocks through activating the glycolytic pathway. Glyceraldehde-3-phosphate dehydrogenase (GAPDH), an integral glycolytic enzyme, is activated upon interaction with GSTP1. As a result, production of ATP and cellular constituents is escalated.

We are studying the effects of GSTP1 knockdown on a panel of PDAC cell lines with different metabolic needs. Our results clearly show the dependency of a glycolytic PDAC cell line on GSTP1 for growth and survival. Our preliminary data also indicate enhanced sensitivity of glycolytic cancer cells towards an ROS inducing agent piperlongumine (PL). PL physically binds to and inhibits GSTP1. Interestingly, we have found PL is not as cytotoxic to cells with reduced GSTP1 levels, which indicates PL primarily works by inhibiting GSTP1 activity. Together, these data suggest GSTP1 is a therapeutic target for PDAC.

Anti-RAGE Antibody as a Combination Therapy with Gemcitabine for Pancreatic Cancer

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PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
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Pancreatic cancer (PC) is a lethal disease with unceasingly growing rate of incidence. In most cases, PC is diagnosed at advanced disease stages, when resection is not feasible. Gemcitabine has been used as the first line drug for treatment of pancreatic cancer with very modest improvement in patient survival. Investigations are required to determine new targets for development of therapies in combination with gemcitabine to improve its activity. Several reports have shown that the Receptor for Advanced Glycation End products (RAGE) contributes to the progression of pancreatic cancer. RAGE has been reported to promote pancreatic tumor cell survival during chemotherapy by supporting autophagy and limiting apoptosis.

In this study, we intended to determine the efficacy of a monoclonal anti-RAGE antibody (IgG 2A11) alone and in combination with gemcitabine, in a mouse model. We used an orthotopic tumor model in C57Bl/6 mice using syngeneic tumor cells line derived from KPC mice to develop pancreatic cancer. There were four treatment groups: (i) Control IgG and Saline (ii) Control IgG and gemcitabine (iii) IgG 2A11 and Saline (iv) IgG 2A11 and gemcitabine. The tumors obtained from the different treatment groups were assessed for their size and volume. A significant reduction in tumor volume was observed for the mice treated with the combination of gemcitabine and IgG 2A11, compared to the control group (Control IgG and gemcitabine). We will next determine the levels of marker for apoptosis (cleaved caspase 3), cell survival (Bcl-2), cell proliferation (Ki67) and autophagy (LC3) in the tumor tissues from the different mouse groups.

Over-expression of RAGE Leads to Proliferative Phenotype in PANC-1 Pancreatic Cancer Cells

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Pancreatic cancer is characterized by late diagnosis, aggressive clinical course and resistance to existing therapies making it a significant reason for cancer mortality. Development of early diagnostic strategies and effective treatment is important for improving patient survival. Recent efforts have concentrated on identifying new targets and molecular pathways which contribute to the development and progression of this lethal disease, and which could be used to establish new therapeutic approaches.

The Receptor for Advanced Glycation End products (RAGE) contributes to the progression of pancreatic cancer. RAGE has been demonstrated to be involved in processes like apoptosis, autophagy, and tumor cell bioenergetics. However, the effect of RAGE expression on the proliferative and migratory properties of pancreatic cancer cells is currently not known. A better understanding of the mechanisms of RAGE signaling could lead to the development of new therapeutic strategies in pancreatic cancer.

Our objective was to investigate the effect of RAGE up-regulation on the proliferation and migration properties of the PANC-1 pancreatic cancer cell-line. We generated cells that overexpress RAGE four-fold compared to non-transfected cells. We then compared the proliferation and migration properties of the new cells with those of the wild-type cells. We also compared the signaling pathways affected by RAGE overexpression in these cells.

We showed that four-fold up-regulation of RAGE in PANC-1 cells resulted in increased cell proliferation at the expenses of cell migration. Both Boyden chamber and wound healing supported the reduced migratory properties of the RAGE overexpressing cells. Further analysis showed significantly reduced levels of integrins in the RAGE transfected cells. We also observed significant reduction of phosphorylated FAK and Akt in the RAGE overexpressing cells as compared to wild-type cells. We suggest that in the PANC-1 cells, which exhibit a mixed epithelial–mesenchymal phenotype, RAGE expression switches the phenotypic balance toward a proliferative phenotype.

Activation of RAGE by AGEs in Pancreatic Cancer Cells

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Pancreatic cancer is the fourth leading cause of cancer deaths in the United States. Although new treatment options have been proposed to patients, their five-year survival rate remains very low and a better understanding of pancreatic cancer development and progression is necessary. Recent studies have suggested that the Receptor for Advanced Glycation End Products (RAGE) contributes to pancreatic cancer growth and metastasis formation by increasing autophagy, cell survival and by decreasing apoptosis. My project aims at investigating how activation of RAGE by Advanced Glycation End Products (AGEs) influences cancer cell growth.

In this study, we investigated how AGEs affect the proliferation of four KPC derived cell lines. These cell lines derive from KPC tumors, and were obtained from the University of Nebraska in Omaha. The cells were stimulated with a series of concentration of AGEs and changes in cell proliferation were determined using the redox dye Alamar Blue. As part of the study, we also determined the levels of RAGE in these cells in order to correlate AGE-stimulated cell proliferation to RAGE levels in these cells.

Delta-5-Desaturase Knockdown and DGLA Supplement Inhibit Colon Cancer Growth

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As essential Ð-6 fatty acids, dihomo-Ð-linolenic acid (DGLA) and its downstream arachidonic acid are both substrates of Cyclooxygenase-2 (COX-2), which is commonly overexpressed in colon cancer. In contrast to arachidonic acid, which promotes colon cancer growth by producing deleterious metabolites via COX-2-catalyzed peroxidation, our previous study showed that DGLA can be metabolized by COX-2 to produce a distinct byproduct, 8-hydroxyoctanoic acid, which actually possess anti-cancer activity. We thus hypothesize that, by knocking down delta-5-desaturase (D5D, a key enzyme that converts DGLA to arachidonic acid), the highly expressed COX-2 in cancer cells can be taken advantage to promote DGLA peroxidation and thereby to elicit anti-cancer activity.

PANCREATIC CANCER AND RELATED DISEASES SYMPOSIUM
NORTH DAKOTA STATE UNIVERSITY

In our present study, D5D knockdown (via siRNA or shRNA transfection) along with DGLA supplement inhibited growth of human colon cancer cell (HCA-7) both in vitro and in a mice xenograft tumor model. A significant accumulation of 8-hydroxyoctanoic acid was observed in D5D knockdown cells/tumors treated with DGLA. In addition, our strategy also greatly enhanced the efficacies of various chemo-drugs, associated with further activated of apoptotic pathway. For the first time, we demonstrated that D5D knockdown is an effective strategy to elicit DGLA's anti-cancer activity; and that the overexpressed COX-2 in cancer cells can be taken advantage to control cancer cell growth, which represents a paradigm shifting concept in contrast to the COX inhibition strategy. This work is supported by NCI/NIH: R15CA140833 and Sanford Health-NDSU Collaborative Research Seed Grant

Knocking Down Delta-5 Desaturase Can Inhibit Growth of Pancreatic Cancer Cell and Tumor with Overexpressed COX-2

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Our recent research has shown that knockdown of delta-5-desaturase (D5D, a key enzyme that converts dihomo-Ð-linolenic acid, DGLA to arachidonic acid) promoted formation of an anti-cancer byproduct 8-hydroxyoctanoic acid (8-HOA) from COX-catalyzed DGLA peroxidation. 8-HOA can exert its growth inhibitory effect on colon cancer cells by serving as a histone deacetylase inhibitor. Here, we extended this novel research finding for inhibiting pancreatic cancer cell and tumor growth, as COX-2 also commonly overexpresses in the specimens of pancreatic cancer patients. Wild-type and D5D knockdown via siRNA/shRNA transfection BxPC-3 (human pancreatic cancer cell line with high COX-2 expression) were used in our study. Tumor xenografts were established by injecting D5D knockdown or wild-type BxPC-3 cells into the flank area of the mice. Clonogenic assay, FITC-Annexin V/PI double staining, PARP staining, and western blot were used to assess cancer cell and tumor proliferation, apoptosis and associated molecular mechanisms. Formation of 8-HOA was detected by GC/MS in cancer cell and tumors.

Our results showed that knockdown of D5D and DGLA supplement not only significantly inhibits cancer cell and tumor growth but also improves the efficacies of gemcitabine, a frontline chemotherapy drug currently used in pancreatic cancer treatment. The molecular mechanism behind these observations is that 8-HOA inhibits histone deacetylase and cause DNA damage, resulting in downregulation of anti-apoptotic protein, e.g. Bcl-2 as well as activation of pro-apoptotic proteins, e.g. procaspase-3 and procaspase-9. For the first time, we demonstrated that we could take advantage of the overexpressed COX-2 level in cancers (common phenomenon) to inhibit pancreatic cancer cell and tumor growth. With shifting paradigm of COX-2 biology in cancer treatment, the research outcome may provide us a novel cancer treatment strategy or a Ð-6s-based diet care to supplement current chemotherapy. This research is supported by NIH R15CA195499-01A and NDSU-Sanford Collaborative Research Seed Grant.

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