Fall 2011

Walther Cancer Foundation Symposium

November 13, 14, 2011

Hosted by:

Purdue University

Center for Cancer Research

Fall 2011 Walther Cancer Foundation Symposium
Hosted by Purdue University, West Lafayette, IN
November 13-14, 2011

November 13, 2011
11/13/2011

4:00 p.m. – 5:00 p.m.Registration *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

5:00 p.m. – 5:30 p.m.Welcome, introductory remarks
Walther Cancer Foundation, Purdue Leadership, Organizing Committee *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

5:30 p.m. – 6:30 p.m. Plenary Session I: Keynote Address:
Tom O'Halloran, PhD - *Therapeutic Efficacy of Nanoscale Arsenic-Platinum Agents in Murine Models of Triple Negative Breast Cancer*

6:30 p.m. – 7:30 p.m.Opening Reception *(West Faculty Lounge, Purdue Memorial Union)*

7:30 p.m. – 9:00 p.m.Dinner *(West Faculty Lounge, Purdue Memorial Union)*

November 14, 2011

11/14/2011
7:00 a.m. Registration *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

7:00 a.m. – 7:45 a.m. Continental Breakfast *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

7:45 a.m. – 10:40 a.m. Plenary Session II: Drug Development and Cancer Biology *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

Chair M. Sharon Stack
Co-chair Don Bergstrom

8:00 a.m. – 8:25 a.m. Andy Mesecar – *Structural and Chemical Mechanisms of Natural Product Cancer Chemoprevention*

8:30 a.m. – 8:55 a.m. Amanda Hummon - *Proteomics Examination of Colon Cancer*

9:00 a.m. – 9:15 a.m. Break

9:15 a.m. – 9:40 a.m. Don Bergstrom - *Self-Assembling Drugs*

9:45 a.m. – 10:10 a.m. Tracy Vargo-Gogola - *Transgenic murine models to investigate role of Rho GTPases in mammary development and cancer*

10:15 a.m. – 10:40 a.m. Dave Miller - *RNA-seq whole transcriptome analysis of cancer*

11:00 a.m. – 12:30 p.m. **Poster Session/Coffee Break** *(East Faculty Lounge, Purdue Memorial Union)*

12:15 p.m. – 1:30 p.m. **Lunch** *(West Faculty Lounge, Purdue Memorial Union)*

1:45 p.m. – 4:00 p.m. Plenary Session III: Translational Cancer Biology *(Pfendler Hall, Dean's Auditorium, PFEN 241)*

Chair Murray Korc
Co-chair Kenneth P. Nephew

1:45 p.m. – 2:10 p.m. Kenneth P. Nephew - *Epigenetic resensitization of solid tumors*

2:15 p.m. – 2:40 p.m. Jamie Renbarger : *Optimizing use of vincristine for children with cancer*

2:45 p.m. – 3:10 p.m. Murray Korc: *Pancreatic Cancer: The need for a new research paradigm*

3:15 p.m. – 3:40 p.m. Gabriela Chiorean – *"From Bench to Bedside: A Clinician's Look into Cancer Biology"*

3:40 p.m. – 4:00 p.m. Final Comments-Adjourn

Poster Abstracts

Posters will be displayed in numerical sequence as listed below:

Poster #	Poster Presenter	Institution
1	Reserved	
2	Scott Crist	Purdue University
3	Marietta Harrison	Purdue University
4	Kevin Kerian	Purdue University
5	Shuhua Yue	Purdue University
6	Christina Ferreira	Purdue University
7	Mini Thomas and Alexander Wei	Purdue University
8	Shawn Liu	Purdue University
9	Christopher Suarez	Purdue University
10	Fang Fang	Indiana University
11	David Lewis	IUPUI
12	Yuliya Klymenko	University of Notre Dame
13	Jian Mu	University of Notre Dame
14	Kara Huegel	University of Notre Dame
15	Jeanette Young	University of Notre Dame
16	Joseph Arico	University of Notre Dame
17	Kristen Alexander	University of Notre Dame
18	Xiaoshan Yue	University of Notre Dame
19	Ana Jakimenko	University of Notre Dame
20	Lance Hellman	University of Notre Dame
21	Serhan Turkyilmaz	University of Notre Dame

CHOLESTEROL SULFOTRANSFERASE (SULT) 2B1B: A NOVEL REGULATOR OF MALIGANT PHENOTYPE IN PROSTATE CANCER.

Scott Crist , Livia Eberlin, Graham Cooks, Timothy Ratliff and ChangDeng Hu

Departments of Comparative Pathobiology, Chemistry, Medicinal Chemistry and Molecular Pharmacology and the Purdue University Center for Cancer Research, Purdue University, West Lafayette, IN 47907

Cholesterol is established as an important mediator of the malignant phenotype in prostate cancer (PCa). The cholesterol derivative cholesterol−3− sulfate (CS), is uniquely accumulated in neoplastic lesions but not in normal epithelial cells in human prostate specimens. CS was also expressed in the human prostate cell line LNCaP, indicating increased expression or activity of cholesterol sulfotransferase (SULT) 2B1b, a member of a diverse family of enzymes that modulate sterol function. Other putative functions of SULT2B1b include regulation of Liver X receptor (LXR) pathway, an established modulator of proliferation, lipid metabolism, and androgen responses in PCa. The differential expression of CS in prostatic neoplasia versus normal epithelium prompted studies to evaluate the role of SULT2B1b on the malignant phenotype in PCa. We correlated CS and SULT2B1b expression in the human prostate cell lines using absorption electrospray ionization (DESI) and qRT-PCR, respectively. We modulated endogenous SULT2B1b expression in LNCaP using lentivirus-delivered short interfering RNA (siRNA). Alteration in basal and androgen- stimulated proliferation rates were determined using standard in vitro growth assays. Androgen responses were assessed by PSA expression, alterations in proliferation, and transcription of transfected co androgen−responsive (ARRE) luciferase reporter plasmids. LXR transcriptional activity was determined by activity of LXR-responsive reporter plasmids and by modulation of LXR target genes. All gene expression profiles were determined using qRT−PCR and TaqMan primers and probes. Our results show SULT2B1b expression correlates with CS production and was limited to LNCaP but not other androgen-independent PCa cell lines PC3, DU145, and ALVA31. Suppression of endogenous SULT2B1b reduced LNCaP basal growth rate, diminished basal and androgen-induced PSA expression, and reduced transcription of the ARRE-luciferase constructs. Conversely, SULT2B1b knockdown enhanced LXR transcriptional activity and expression of LXR−dependent SREPB−1c and ABCG1 expression. Overall, the data show CS production correlates with SULT2B1b expression and that modulation of SULT2B1b impacts malignant phenotype in PCa. The data also show that SULT2B1b modulation directly impacts LXR activity, suggesting these phenotypic alterations may be mediated by SULT2B1b−mediated inactivation of endogenous LXR agonists. Overall, these data support a novel role for SULT2B1b as an important modulator of several biological functions in PCa.

Cancer Care Engineering

Patrick J. Loehrer, MD, Marietta L. Harrison*†, PhD, Julie S. Nagel*, PhD, Joseph F. Pekny*‡, PhD,
Indiana University Melvin and Bren Simon Cancer Center, *Purdue University Oncological Sciences Center, †Department of Medicinal Chemistry and Molecular Pharmacology, ‡School of Chemical Engineering, Purdue University

Cancer Care Engineering (CCE) is a large-scale, multi-institutional, discovery-based, interdisciplinary project that applies the principles of systems engineering to the prevention, early detection, and effective treatment of cancer, using colorectal cancer as an initial focus. A defining and unique feature of the clinical-based arm of CCE is the integration of data from multiple biological analyses performed on a single blood and/or tissue sample derived from an individual patient. The project is driven by a medical oncologist, a biochemist and a systems engineer and is overseen by a dedicated project manager. Since accrual started in April 2009, over 250 samples have been collected from normal, polyp bearing, and cancer patients undergoing screening and diagnostic colonoscopic examination n the academic-based clinics at the Indiana University Cancer Hospital. Collection at a second, community-based hospital site is schedule to begin in the late fall of 2011. Blood samples are simultaneously assayed for global and selected proteins, global and selected metabolites, selected lipids, and status and polymorphisms for oxidative stress, vitamin D and DNA repair, while tissues are analyzed for gene expression, selected polymorphisms/mutations, chromosomal instability, microsatellite instability, and methylation status. Data are integrated and mined for differential patterns to identify and segregate populations using statistical modeling and visualization analytics. This effort is supported by a cyber infrastructure based on Purdue's powerful HUBzero™ technology where annotated, analyzed data are stored, handled, queried, modeled and visualized in a single, easily accessible environment. The cceHUB.org provides the large-scale data storage capacity and data mining capability demanded by the CCE project and enables instantaneous communication between the clinicians, scientists, statisticians, and engineers who are working with the massive datasets generated by the CCE project. The defining molecular signatures of the segregated populations will be used to generate novel screening, risk assessment and decision making tools to optimize and personalize cancer prevention and therapy. The CCE team includes over fifty professionals from Indiana University, Purdue University, the University of Notre Dame, the Roudebush VA, and the MD Anderson Cancer Center and is supported by the DOD, Regenstrief Foundation and the Walther Cancer Foundation.

CHOLESTEROL SULFATE IN HUMAN PROSTATE CANCER INVESTIGATED BY DESI-MS

Kevin Kerian, Livia S. Eberlin, and R. Graham Cooks. Department of Chemistry, Purdue University, West Lafayette, IN 47907.

Cholesterol sulfate (CS) was previously found to be a possible marker for prostate cancer by desorption electrospray ionization mass spectrometry (DESI-MS)[1]. CS has been observed at increased relative intensity in cancerous tissues or tissue containing precancerous lesions, while usually being undetected in adjacent normal tissue. In order to further investigate the effectiveness of CS as a potential tissue prostate cancer marker, studies were expanded to high grade prostate cancer tissue and metastatic prostate cancer tissue. DESI-MS imaging was used to analyze high Gleason score prostate tissue in negative ion mode. CS (m/z 465.4) was shown to have similar intensities to that of other phospholipids in the mass spectra, such as PS(36:1), m/z 788.3, and PI(38:4), m/z 885.4 for all of the sample analyzed. Trends in CS intensities correlated to Gleason scores are being investigated together with detailed histopathological evaluation of the same tissue section after H&E staining. In metastatic tissue samples, CS was not detected in the majority of tissue analyzed, giving evidence of a relationship between androgen dependency and CS expression. The usefulness of CS as a non-invasive marker of prostate cancer was also evaluated through the analysis of biofluids such as urine and serum. Urine sediment obtained from cancer patients after prostate massage was analyzed by DESI-MS in the negative ion mode. CS was detected in the sediment with similar intensities to other characteristic membrane lipids such as PS(36:1) and PI(38:4). The similarities between the mass spectra from the urine sediment and prostate cancer tissue suggest that prostate cancer cells released due to prostate massage were being analyzed in the sediment. The utility of this methodology for non-invasive prostate cancer detection will be further explored with urine samples from normal human subjects.

1. Livia S. Eberlin, Allison L. Dill, Anthony B. Costa, Demian R. Ifa, Liang Cheng, Timothy Masterson, Michael Koch, Timothy L. Ratliff, and R. Graham Cooks, "Cholesterol Sulfate Imaging in Human Prostate Cancer Tissue by Desorption Electrospray Ionization Mass Spectrometry", *Analytical Chemistry*, 2010, 82, 3430-3434

Label-free Spectroscopic Imaging of Lipid Stores in Human Prostate Cancer

Shuhua Yue[1], Liang Cheng[2], Timothy Ratliff[3], Ji-Xin Cheng[1,3*]

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Although intracellular accumulation of neutral lipids was commonly seen in studies of prostate cancer cell lines, its clinical relevance to prostate cancer risk is not established without knowing the nature of lipid stores in human prostate cancer tissue. By label-free Raman imaging of human prostate tissues, we found lipofuscin-like structures in benign prostate gland, and cholesterol ester-rich lipid stores in prostate cancer with a positive correlation with Gleason score. In vitro, a transition from fat bodies to cholesterol ester–rich lipid bodies is found during proliferation of LNCaP Cells. Moreover the cells with cholesterol ester-rich lipid stores show significantly higher proliferation rate compared to the cells with triglyceride-rich lipid stores. These results show an essential role of cholesterol ester in prostate cancer progression. Our data also suggest that spectroscopic imaging of lipid stores could potentially be used for differentiating low grade and high grade prostate cancer.

Chemistry of Cellular Pluripotency using Desorption Electrospray Ionization Mass Spectrometry

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Lipids and metabolites play fundamental biological roles in controlling biochemical pathways in terms of cell fate and differentiation. The complex processes and mechanisms involved in cellular pluripotency are poorly understood and represent a recent area of active exploration, especially in cancer stem cell research. We have developed an analytical strategy based on desorption electrospray ionization (DESI-MS) to perform rapid chemical analysis of microscopic samples in different stages of pluripotency and differentiation, represented by mouse single oocytes and preimplantation embryos. The DESI-MS approach involves the use of recently introduced solvent systems based on dimethylformamide (DMF), which are able to gradually extract the lipids and other small molecules from intact organisms and to accumulate enough signal to generate good quality mass spectra. DESI-MS analysis was performed on mouse oocytes and embryos from different phases of pre-implantation development EtOH/DMF or ACN/DMF 1:1 (v/v) as solvents. Both solvent combinations were successful and very similar distributions of lipid species were observed. Free fatty acids (FA) occurred in the lower mass range (m/z 200 - 350), FA dimers in the region of m/z 500-650 and glycerophospholipids in the m/z 700 – 1000 range. Deprotonated free FA represented included palmitic (m/z 255.2), linoleic (m/z 281.2), arachidonic (m/z 303.2) and docosahexanoenic (m/z 327.2), and prominent fatty acid dimers included the oleic acid dimer at m/z 563.5. Complex lipids were represented by phosphocholines (such as PC 34:1, m/z 794.5), phosphatidylinositols (such as PI 38:4, m/z 885.8), phosphatidylethanolamines (such as PE 38:3, m/z 768.5) and phosphatidylserines (such as PS 38:4, m/z 810.7). The long-lasting lipid signals allow tandem MS experiments to be performed for lipid characterization. Our current experiments aim at analyzing a significant set of mouse oocytes and embryos in diverse developmental stages (2-cell, 8-cell, morula and blastocyst) recovered in vivo or cultured in vitro and the respective derived embryonic stem cells in order to characterize the lipid signatures from the different stages of pluripotency and cellular differentiation and to evaluate the effect of in vitro culture on the lipid composition of embryos. Small molecules and such lipids such as retinoic acid, can direct cellular differentiation. It is known that this process is accompanied by intense gene expression and protein synthesis activity, but lipid roles are less explored. We envisage that lipidic structural information provided by DESI-MS analysis of embryos and embryonic stem cells will complement data on gene and protein expression and will provide basic information relevant to the area of cancer stem cell research.

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Light-activated nanorods for siRNA therapy of ovarian cancer

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Ovarian cancer (OC) is the fifth leading cause of cancer death in the United States, with an estimated 25,000 new cases of ovarian cancers and 14,000 deaths annually. Recent studies indicate that the metastatic progression in ovarian cancer may be critically dependent on an enzyme known as TG2. This enzyme has been linked to a number of biochemical processes deemed essential to tumor development and also to tumor cell survival. TG2 is a promising target for a new type of therapy, in which small pieces of RNA (siRNA) can be used to silence the production of TG2, thereby preventing further progression in tumor metastasis. In this project, we propose to develop siRNA carrier systems that use gold nanorods (GNRs) as light-activated "lancets" to deliver siRNA cargo into ovarian cancer cells. GNRs can convert near-infrared light into local bursts of energy, enabling them to inject siRNA through cell membranes and shut down TG2 production and hence reduce their metastatic progression.

POLO-LIKE KINASE 1 FACILITATES LOSS OF PTEN-INDUCED PROSTATE CANCER FORMATION

X. Shawn Liu[1, 3], Bing Song[2, 3], Bennett D. Elzey[3, 4], Timothy L. Ratliff[3, 4],
Stephen F. Konieczny[2, 3], Liang Cheng[5], and Xiaoqi Liu[1, 3]
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Loss of the tumor suppressor Pten (phosphatase and tensin homolog deleted on chromosome 10) is thought to mediate the majority of prostate cancers, but the molecular mechanism remains elusive. In this study, we demonstrate that Pten-depleted cells suffer from mitotic stress, and that nuclear function of Pten, but not its phosphastase activity, is required to reverse this stress phenotype. Further, depletion of Pten results in elevated expression of Polo-like kinase 1 (Plk1), a critical regulator of the cell cycle. We show that overexpression of Plk1 correlates with genetic inactivation of Pten during prostate neoplasia formation. Significantly, we find that elevated Plk1 is critical for Pten-depleted cells to adapt to mitotic stress for survival, and that re-introduction of wild-type Pten into Pten-null prostate cancer cells reduces the survival dependence on Plk1. We further show that Plk1 confers the tumorigenic competence of Pten-deleted prostate cancer cells in a mouse xenograft model. These findings identify a role of Plk1 in facilitating loss of Pten-induced prostate cancer formation, which suggests that Plk1 might be a promising target for prostate cancer patients with inactivating Pten mutations.

Targeting CREB signaling as a strategy for prostate cancer radiotherapy

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Despite significant advances in better treating and managing prostate cancer, challenges remain in understanding and developing treatments for recurrent onset of the disease. One possible mechanism, involving the pathway to recurrence, is the transdifferentiation process, by which prostate cancers differentiate into neuroendocrine-like cells, also known as neuroendocrine differentiation (NED). NED has been associated with progression of prostate cancer, androgen-independent growth and poor prognosis. We previously reported that fractionated ionizing radiation (IR) induces NED. Dedifferentiation of these neuroendocrine-like cells gives rise to a population that has become resistant to chemotherapeutic agents and further IR induced cell death. In addition, we found that IR induced NED is associated with increased CREB phosphorylation. To examine the role of CREB in IR-induced NED, two approaches were utilized. A lentiviral short-hairpin (shRNA) approach was utilized to create tetracycline-inducible stable cells lines knocking down CREB and a separate tetracycline-inducible expression system was utilized to express a dominant negative CREB named acidic CREB, or ACREB. We found that knockdown of CREB in LNCaP cells decreased IR-induced neurite extension and the percentage of surviving cells after a 40 Gy cumulative dose. Likewise, expression of ACREB increased sensitivity to IR after a fractionated dose of 10Gy. To determine the mechanism underlying ACREB-induced cell death, we performed Annexin-V staining and found a 16% increase in Annexin-V positive cells after 6Gy IR. Additionally, we observed that IR treatment of LNCaP cells can be broken down into two unique phases. The first phase occurs during the inital two weeks of fractionated IR treatment and is associated with the acquisition of radioresistance. During the first two weeks, approximately 80% of the cells are killed with approximately 20% remaining viable. Only those cells able to acquire radioresistance progress to the next phase involving the acquisition of NED. Interestingly, knockdown of CREB was sufficient to inhibit IR-induced neurite extension, but did not have an apparent effect on IR-induced cell death during the first two weeks. On the other hand, expression of ACREB, which can bind all CREB/ATF1 family transcription factors and almost completely knockdown CREB expression, sensitized 34% of cells to IR during the first week. Taken together, we provide evidence that targeting CREB signaling, by inhibiting both the radioresistance and NED phases, is an attractive strategy for prostate cancer radiotherapy.

SYSTEMS BIOLOGY IDENTIFICATION OF PROCESSES/PATHWAYS ORIGINATING HIGH-GRADE SEROUS EPITHELIAL OVARIAN CANCER.

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Previously, based on well-known stem cell phenotypes (sphere formation, potent tumorigenicity, embryonic gene expression, chemoresistance), we used patient tumors to isolate high-grade serous (HGS) ovarian cancer-initiating cells (OCICs), expressing the cell surface markers CD44 (a hyaluronate receptor) and CD117 (C-KIT) (Zhang *et al.*, 2008, *Cancer Res* 68:4311). Here, to identify biological pathways/processes responsible for facilitating OCIC tumorigenic potency and self-renewal, we performed integrated analyses of global gene expression (Gene 1.0 ST, Affymetrix), DNA copy number (Human CGH 3x720K Whole-Genome v3.0, NimbleGen), and DNA methylation (Infinium Human Methylation27 BeadChip, Illumina) patterns, as compared to "bulk" (*i.e.*, disaggregated but unsorted) tumor cells. OCICs and bulk tumor cells were also compared to primary human normal ovarian surface epithelial (NOSE) cells that, along with fallopian tube epithelial (FTE) cells, represent hypothesized cells-of-origin for HGS pelvic cancers. Principle components analysis and unsupervised hierarchical clustering revealed distinct DNA methylation and gene expression profiles for replicate (n=3) samples of each cell type (*i.e.*, OCIC, bulk tumor, and NOSE). Gene ontology and additional unsupervised analyses of "gene expression signatures" of the epithelial-to-mesenchymal transition (82 of 159 signature genes), TGF-beta (47 of 86 genes), and Wnt (75 of 151 genes) demonstrated distinct segregation of the three cell types, with mesenchymal and Wnt signaling hierarchies being NOSE > OCIC > bulk tumor cells. To assess possible early events in tumorigenesis, public gene expression datasets were employed to show that NOSE, OCIC, and bulk tumor cell transcriptomes distinctly segregated from normal and malignant FTE cell transcriptomes. Interestingly, the largest divergence was between the NOSE and normal FTE cell gene expression profiles. Finally, gene expression and DNA methylation patterns were integrated, implicating specific networks/circuits in ovarian carcinogenesis. Taken together, these results support a sequentially increased mesenchymal-to-epithelial transition during ovarian tumor progression, reveal legitimate therapeutic targets (at the pathway level), and raise uncertainty that surface epithelial cells of the fallopian tube and ovary are reciprocal cells-of-origin for HGS pelvic carcinoma.

ENHANCING ANTI-TUMOR IMMUNITY OF NK CELLS BY SOYPEPTIDE

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Natural killer (NK) cells are important components of the innate immune system and play a major role in the rejection of tumors and virus infected cells. By producing proinflammatory cytokines such as IFNgamma, NK cells directly exert immunoregulatory functions that influence adaptive immunity by modulating the activity of other immune cells. Given its critical role as guardian for health, interventions to manipulate NK activity has been pursued by using various cytokines including IL-12 and IL-2. NK cells respond to cytokines in a dose-dependent manner; however, the toxicity of certain cytokines (e.g. IL-2) in high doses prohibits their systemic administration. Therefore, novel strategy to activate NK cells with less toxicity is warranted. We recently exploited a natural dietary peptide called lunasin to improve the immune functions. The research objective is to test the hypothesis that lunasin peptide has immunomodulatory effects on immune cells. To test this hypothesis, human peripheral blood mononuclear cells (PBMCs) of healthy donors were stimulated with or without lunasin in combination with or without cytokine IL-12 or IL-2. Our results showed that lunasin peptide exerts robust synergistic effects imposed by the selected cytokine to regulate expression of a number of genes that are important for NK activity. Our findings support the potential clinical use of lunasin in combination with cytokine to enhance anti-tumor immunity of NK cells.

ROLE OF N-CADHERIN EXPRESSION IN OVARIAN CARCINOMA CELL AND MULTICELLULAR AGGREGATE PHENOTYPE

Yuliya Klymenko, and M. Sharon Stack. Harper Cancer Research Institute, University of Notre Dame, Notre Dame, IN, 46566

Epithelial Ovarian Carcinoma (EOC) is the fifth leading cause of women's cancer-related death and deadliest of all gynecological cancers primarily due to detection on late metastatic, prognostically poor stages of the disease. In contrast to most tumors, EOC demonstrates a unique metastasizing mode, which includes shedding of epitheliocytes from primary carcinoma into peritoneal cavity, further dissemination in form of single cells and multicellular aggregates (MCAs) with subsequent intraperitoneal adhesion, migration through mesothelial layer and invasion of submesothelial matrix to form secondary tumor masses. Another distinct feature of EOC is increase of E-cadherin expression at early stages of metastasizing, frequently together with conserved level of N-cadherin. cDNA microarray analysis and dual label immunofluorescent microscopy of tissue array confirm simultaneous expression of both E- and N-cadherins in majority of ovarian cancer cells as oppose to normal ovarian epithelium expressing N-cadherin only. The mechanisms providing initial cell detachment, MCA generation, survival in ascitic fluid and secondary anchorage as well as role of E- and N-cadherin in these steps remain poorly understood. Our previous research has revealed dramatic differences in MCA formation, surface morphology and inner ultrastructure, cohesion and stability depending on cadherin type expression of composing cells, varying from highly cohesive smooth solid spheroids (DOV13 Ncad+ cells) to loosely conglomerated and easy-breaking aggregates (OvCa433 Ecad+ cells). To further investigate the role of cadherins we have generated a stable "true hybrid", bicadherin expressing OvCa433 Ecad+/Ncad+ cell line. Scanning electron microscopy demonstrated relevant changes in MCA surface morphology with increased amount of laminar-like structures (lamellipodia) and short filaments (filopodia) in OvCa433Ecad+/Ncad+ MCAs compared to OvCa433Ecad+ control MCAs, uniformly covered by microvilli. Our studies also showed enhanced invasive properties of OvCa433Ecad+/Ncad+ line in cell migration and Matrigel invasion assays. Dispase-based dissociation of a hybrid Ecad+/Ncad+ cell monolayer resulted in a longer time period in dispase needed to detach together with higher number of segregated fragments compared to Ecad+ control. These data suggest the impact of N-cadherin on EOC cell and MCA properties with potential contribution to EOC progression.

QUANTITATIVE ANALYSIS OF SPATIAL DISTRIBUTION OF CELLS IN BREAST CANCER.

Jian Mu, Danny Chen, Department of Computer Science and Engineering, University of Notre Dame, Notre Dame, IN 46556

Nupur Bhattacharya, Peter P. Lee, Medical School, Stanford University, Stanford, CA 94305

It is found that distribution of immune cells (for example, dendritic cells, T cells, etc.) and tumor cells in lymph nodes of breast cancer patients is related to the stage of the disease and clinical outcome. In this research, we aim at quantitatively studying the spatial distribution of cells in lymph nodes. We propose a density-based clustering algorithm to identify clusters of dendritic cells, and then study the distribution patterns of other cells, such as T cell-DC interactions and interactions between tumor and the immune system based on the clustering results. We further proposed hypotheses to explain the cell distribution behaviors. This research may help reveal the interplay between cancer and the immune system and is of considerable biomedical significance. This analysis may also be important to the development of therapeutic strategies for treating cancer.

A HIERARCHY OF KINASE-DRIVEN INTERACTIONS REQUIRED FOR CHECKPOINT SIGNALING DURING MITOSIS

Kara L. Huegel and Kevin T. Vaughan.

Department of Biological Sciences, University of Notre Dame, Notre Dame, IN 46556

The spindle assembly checkpoint (SAC) is responsible for resisting anaphase onset during prometaphase but also for triggering anaphase onset once chromosome alignment has been achieved. The mechanisms that integrate these two questions into a single response are not known. The Vaughan laboratory has investigated phosphorylation of cytoplasmic dynein at kinetochores as an indicator of progression through chromosome alignment and as a sensor of chromosome alignment. Based on the identification of PP1 as a dynein phosphatase at metaphase, we used small molecule inhibitors and chemical genetics to identify mitotic dynein kinases. Inhibition of Plk1 with BTO-1 or analogue-sensitive Plk1 constructs ablated recruitment of phospho-dynein to kinetochores and induced errors in chromosome alignment during prometaphase. Dynein-binding proteins implicated in recruiting kinetochore dynein were not affected. In parallel, we assessed the requirement for Aurora B (AurB) in dynein recruitment, based on the antagonistic roles of PP1 phosphatases and AurB homologues in yeast. Inhibition of AurB blocked recruitment of phospho-dynein to kinetochores. However, the effects on phospho-dynein recruitment were indirect. Dynein, dynactin, spindly and *zw10* were each reduced after AurB inhibition, whereas zwint-1, Hec1 and Knl1 were not affected. Because the interaction between zwint-1 and *zw10* has been implicated in recruiting the *rod-zw10*-zwilch (RZZ) complex to kinetochores, we compared phosphorylation of zwint-1 and *zw10* using *in vitro* kinase assays. Zwint-1 but not *zw10* was phosphorylated by AurB, and a set of three novel AurB phosphorylation sites was mapped in zwint-1 by MS/MS analysis. A triple-A zwint-1 mutant blocked recruitment of the RZZ complex and all RZZ-dependent proteins to kinetochores and induced prometaphase arrest. A triple-E zwint-1 mutant overcame the effects of AurB inhibition on kinetochore assembly. However, the triple-E mutant blocked dynein-driven streaming of checkpoint proteins at metaphase, inducing metaphase arrest. These results suggest that phosphorylation of zwint-1 by AurB is required for assembly of a dynein-binding platform at kinetochores during prometaphase. However, dephosphorylation of zwint-1 at metaphase defines the boundary between stable and streaming proteins implicated in checkpoint silencing. These studies clarify the roles of Plk1 and AurB in the regulation of kinetochore dynein.

Identification of the Biosynthetic Gene Cluster for the Cytostatic Polyketide Gephyronic Acid from *Cystobacter* strain cbvi 76

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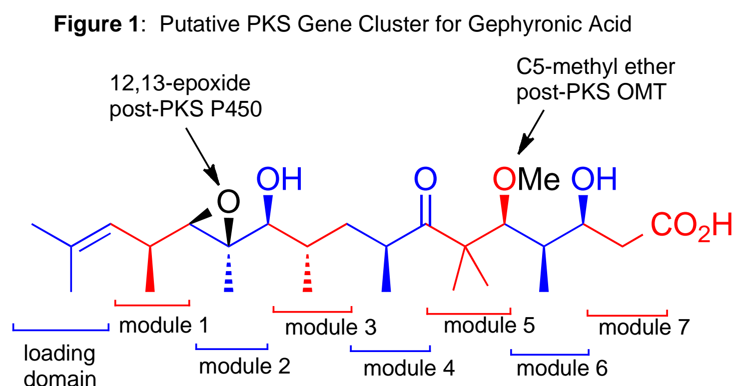
Gephyronic acid is an antibiotic natural product isolated at the HZI (Helmholtz-Zentrum für Infektionsforschung) by Sasse and Höfle from the myxobacterium *Archangium gephyra* in 1995. Gephyronic acid has been shown to inhibit the growth of yeast and mold in the micromolar range; it also exhibits a cytostatic effect on mammalian cell cultures (human cervix, human leukemia, human breast) in the nanomolar range. An in vitro translation assay demonstrated that gephyronic acid is a specific inhibitor of eukaryotic protein synthesis. Our recent total synthesis of gephyronic acid incorporates a synthetic route that provides a means by which to prepare gephyronic acid efficiently and selectively and has provided significant quantities, thus facilitating further biological evaluation towards identifying gephyronic acids specific mode of action in cancer cells. Moreover, we seek to identify and characterize the polyketide synthase (PKS) gene cluster responsible for gephyronic acid production in the producing organism. Our proposed biosynthetic pathway involves seven elongation modules and two post-PKS modifying enzymes (figure 1), which has been validated via genetic analysis through complementary use of whole-genome sequencing and the use of a cosmid library derived from the myxobacteria strain *Cystobacter* cbvi 76, another known producer of gephyronic acid. Identification of the gene cluster is the key step in a heterologous expression system, which will provide an alternative source of the natural product and a fermentation system for precursor-directed biosynthesis of gephyronic acid analogues.

Figure 1: Putative PKS Gene Cluster for Gephyronic Acid



14-SUBSTITUTED EPOTHILONE ANALOGUES AS POTENT MICROTUBULE STABILIZING AGENTS FOR CANCER CHEMOTHERAPY

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The recent clinical success of paclitaxel and Ixebepilone (Ixempra) has established microtubule stabilizing agents (MSAs) as powerful chemotherapeutic agents with a unique mode of action. The impressive anticancer activity of these compounds is marred, however, by poor water solubility and nonspecific toxicity, respectively. The epothilones, a small family of polyketide natural products discovered in the mid-1990s, along with hundreds of highly potent analogues including Ixempra, represent important lead structures in the search for potent anticancer compounds with minimal toxicity. Our laboratory has employed computational analysis of the highly flexible macrolactone moiety of the epothilones to identify the likely active conformation, assisting in analogue design. A first-generation set of analogues, containing an additional, stereodefined functional group in the 14-position, exhibited anticancer activity on par with paclitaxel and Ixempra. The nanomolar cytotoxicity of our 14-methoxy epothilone analogue in an ongoing NCI-60 trial motivated us to pursue a second-generation set of analogues with improved activity and, most importantly, favorable pharmacokinetic properties. Ongoing synthetic efforts are aimed at producing significant quantities of several analogues to drive preclinical trials. The synthetic route employed is efficient, scalable, and amenable to the production of multiple analogues from an advanced common intermediate.

Nanoscale Raman Investigation of Adhesion Proteins Relevant to Colon Cancer Metastasis

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Cancer cell metastasis involves changes in the cellular membrane that enable a cell to break away from its origin and spread to a new location in the body. In order for this to occur, chemical changes must take place between the plasma membrane of the cell and the extracellular matrix (ECM). Adhesion proteins, such as integrins and cadherins that hold cells in place, must release and then reattach to enable propagation of cancer cells. Integrins, in particular, have been associated with cancer cell migration and adhesion and serve as promising target molecules to assess changes in the membrane environment of cancer cells before and after metastasis. The goal of this research is to use near-field optical detection of nanoparticles, targeted to integrins on the surface to cells, to enhance the chemical signals relevant to binding and organization of integrins in two colorectal cell lines. The cell lines represent pre- and post- metastasis from a single patient. Near-field detection provides chemical information from molecules in the immediate proximity of the targeted protein. Simultaneous atomic force microscopy and dark-field scattering complement the near-field Raman spectroscopy. It is our aim that, through the development and application of these techniques, we can obtain a better understanding of the role integrins play in the metastasis of cancer.

EFFECTS OF LOW DOSES IRRADIATION ON MAMMALIAN PHOSPHOPROTEOMES

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Cancer arises from insults to the genome, which alters the expression of gene products and causes aberrant abundance patterns of mRNA and proteins. Ionizing radiation (IR) is a well known cause of DNA damage that results in double strand breakage. The DNA damage response pathways are activated mainly through phosphorylation or dephosphorylation of repair enzymes after DNA damage, either to repair the damaged DNA backbone or initiate cell death pathway to deplete the cells carrying mutated genes from the physiological system. Previous studies have reported that high doses of IR exposure cause cell death. We hypothesize that under much lower doses of IR exposure, the damage will initiate the pathways to repair DNA damage. We are using the immortalized mammary cell line, MCF-10A, and exposing them to very low doses of IR (^{137}Cs gamma source, 3.06 Gy/min, total 0.05 Gy). DNA damage is first confirmed by detecting phosphorylation of H2A.X, an indicator of DNA double strand break, via Western blots. Once activation of DNA damage response pathways is detected, we enrich for phosphopeptides after IR using strong cation exchange (SCX) chromatography followed by immobilized metal affinity (IMAC) beads. Global analysis of phosphopeptides is achieved via high-resolution tandem mass spectrometry. By monitoring the changes of phosphoproteomes at different time points after IR, the process of DNA damage repair can be analyzed. Most importantly, we can determine the lowest dose of IR that triggers the DNA damage response pathway, which is useful information for IR safety and cancer treatment.

The Regulation of Anoikis by Carcinoma-Associated Fibroblasts in Breast Cancer Cells

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Carcinoma-associated fibroblasts (CAFs) are now appreciated to play an active role in progression of breast cancer. However, the ability of CAFs to regulate the survival of breast cancer cells is completely uncharacterized. We examined how/if CAFs contribute to the ability of cancer cells to overcome anoikis, a cell death process initiated upon cell detachment from extracellular matrix (ECM). To investigate this question, we used NIH-3T3 mouse fibroblasts lacking the caveolin-1 gene (KO MEFs) as they have been demonstrated to be a good model cell line for human CAFs. We found that addition of media conditioned by KO MEFs led to anoikis inhibition in detached MCF-10A cells when compared with the cell death levels observed in MCF-10A cells grown in media conditioned with wild type (WT) MEFs. We then examined the activation of canonical intracellular signaling pathways following exposure of ECM-detached MCF-10A cells to KO-MEF-conditioned media. Mitochondrial cytochrome *c* release was robustly inhibited in MCF10A cells exposed to conditioned media from KO MEFs. Surprisingly, this inhibition was independent of signaling through PI(3)K, MAPK, or Bim, suggesting a novel mechanism by which KO MEFs inhibit cytochrome *c* release. To examine if the observed differences caused by secreted factors present in conditioned media manifest in a more physiologically relevant context, we employed a 3-dimensional (3D) cell culture model of mammary acinus development. MCF-10A acini exposed to secreted factors from KO MEFs were more prone to have filled lumen than the cells exposed to secreted factors from WT MEFs suggesting that factors secreted from CAFs can promote the survival of ECM-detached cells in the luminal space. In order to validate these data, we obtained diagnostic needle biopsies from breast cancer patients and isolated and cultured human CAFs from them. Despite the inherent variability amongst the samples from the different patients, we have obtained the same results in all cell lines when ECM-detached MCF-10A cells were exposed conditioned media from patient-derived CAFs. Further experiments to identify the factors regulating anoikis that are secreted by KO MEFs and human CAFs are underway.

Engineering T-Cell Receptors to Optimize Anti-Tumor Immunity

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T-cells aid in eliciting an immune response against virally infected cells. They have also been shown to attack and kill various malignant cells. An abnormal cell, also termed the antigen presenting cell (APC), presents an antigen on its surface in complex with the major histocompatibility complex (MHC) proteins. Recognition of malignantly transformed or virally infected cells by T-cells is mediated through the T-cell receptor (TCR). Malignant melanoma is one such malignancy that is immunosensitive. One of the melanoma antigens presented by the MHC is the MART-$1_{27\text{-}35}$ (AAGIGILTV) nonameric peptide, which is recognized by the TCRs DMF4 and DMF5. Clinical trials involving adoptive cell therapy (ACT) of melanoma patients showed cancer regression of 13% and 30% for clonally expanded T-cells genetically engineered to express DMF4 and DMF5 (1,2). Our work involves using structure-guided computational design to enhance the affinity of DMF5 towards the MART-$1_{27\text{-}35}$ peptide, with the eventual goal of assessing the impact of enhanced affinity on anti-tumor immunity in mouse models of melanoma. Thus far, we have generated three higher affinity mutants of DMF5; αD27Y, βL98W, and a double-mutant αD27Y/βL98W, and are optimizing a retroviral expression system to generate gene-modified murine T cells.

1) Morgan RA, *et al*. (2006). Cancer Regression in Patients After Transfer of Genetically Engineered Lymphocytes. *Science*. 314(5796): 126-129

2) Johnson LA, *et al*. (2009). Gene Therapy With Human and Mouse T-Cell Receptors Mediates Cancer Regression and Targets Normal Tissues Expressing Cognate Antigen. *Blood*. 114: 535-546

ENHANCED CELL DEATH IMAGING USING MULTIVALENT ZINC(II)-DIPICOLYLAMINE FLUORESCENT PROBES

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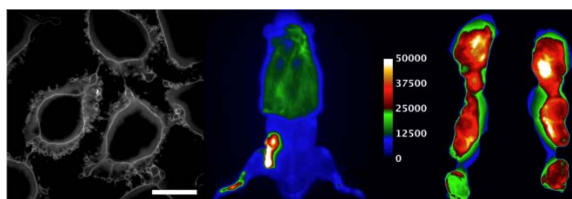
Many drug delivery and imaging probes rely on monovalent recognition elements for high affinity binding to their target receptor, though this desired outcome is not always achieved. One way to increase binding affinity is by designing multivalent probes, where multiple copies of the same ligand are attached and presented from a central scaffold. Multivalent ligand-receptor interactions are important in biological systems for selective cell recognition, cell adherence, and intracellular signalling. During cell death, phosphatidylserine (PS), an aminophospholipid normally secluded to the inner leaflet of the plasma membrane, is translocated to the cell surface providing an estimated 10^9 binding sites for PS targeted molecules. Zinc(II)-bis-dipicolylamine (Zn_2BDPA) coordination complexes have high selectivity towards dead and dying mammalian cells with exposed PS, even though binding affinities of the Zn_2BDPA probes for membrane PS are moderate (micromolar dissociation constants). The likely undesired effects of this modest PS affinity, such as poor target-to-backgraound ratios at low probe doses and possible toxicity due to off-target binding, may prohibit successful translation to the clinic. Here, we show that the fluorescent probe Tetra-Zn_2BDPA-SQR with four attached Zn_2BDPA units has greatly enhanced affinity for cell death compared to analogous probes with fewer Zn_2BDPA units. Cell microscopy studies show that low concentrations of Tetra-Zn_2BDPA-SQR strongly stain the surfaces of dead and dying cells and blebbing apoptotic bodies. Furthermore, in vivo fluorescence imaging studies, using mouse thymus atrophy[1] and rat prostate tumor models[2], found that in vivo uptake of Tetra-Zn_2BDPA-SQR into dead and dying tissue was more than two and four fold than that observed with an analogous probe that has only two Zn_2BDPA units.



Tetra-Zn_2BDPA-SQR shows enhanced staining of apoptotic cell membranes (top left) and localization to the necrotic core of rodent prostate tumors (top middle and right).

[1] Smith, B. A. et al. *Apoptosis* **2011**, doi:10.1007/s10495-011-0601-5
[2] Smith, B. A. et al. *J. Am. Chem. Soc.* **2010**, *132*, 67-69.