Close Window

Title: Predicting Adverse Cardiac Events in Breast Cancer – PACE: Examination of physical activity during chemotherapy. [2014]

Authors: Danielle Berera,[1] Carrie Lenneman,[1] Vasanth Sathiyakumar,[2] Douglas Sawyer.[2] Medicine[1] and Other.[2]

Keywords: PACE

Abstract:

Background:

Breast cancer (BCA) affects one in eight women in the US. Doxorubicin (Dox) and Trastuzumab (Tsz) remain prevalent chemotherapies for breast cancer, but cause cardiotoxicty with significant morbidity and mortality in a subset of patients. The current study is a sub-study of an ongoing prospective observational study investigating if specific cardiac factors, growth factors, genetic polymorphisms and self-reported physical activity can predict which women will develop cardiac dysfunction from chemotherapy. The sub-study of PACE was aimed at characterizing the self-reported physical activity during first three months of chemotherapy.

Methods:

In a prospective, longitudinal study, 132 newly diagnosed breast cancer women receiving either AC or Tsz were enrolled over a 4-year period. Baseline data at enrollment and at 4 time-points during first three-months of chemotherapy were ascertained. Enrolled participants were given baseline physical activity questionnaire and 4 additional CHAMPS questionnaire during chemotherapy. Complete questionnaires from 86 patients were analyzed.

Results:

The mean age of this cohort was 50 years old with a Caucasian predominance. Physical activity significantly decreased in during Dox treatment in compared to TSZ. However looking at the combined group of Dox and TSZ there were a statically significant trend showing an overall decrease in self-reported physical activity.

Conclusions

Women enrolled in PACE have a high number of cardiovascular risk factors. Our study demonstrates that most women describe a decrease in their physical activity during chemotherapy. It remains unknown if an exercise prescription decreases the likelihood of developing cardiotoxicity. Further work is needed with ongoing prospective studies.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=4019

Close Window

Title: Calorimetry of the Plasma Proteome in Patients with Ovarian Cancer [2014]

Authors: MB Hatch,[1] Nichola Garbett,[2] Alagammai Kaliappan.[2] University of Louisville School of Medicine [1] and James Brown Cancer Center.[2]

Keywords: Calorimetry, Proteome, Ovarian, Cancer, Haptoglobin, Albumin

Abstract:

Background/Objective: Ovarian cancer is exceptionally deadly because of the frequent late-stage diagnosis. The goal of this project is to analyze the blood plasma proteome from ovarian cancer patients to discover new aspects of ovarian cancer biology and determine the utility of calorimetry as a possible diagnostic technology.

Methods: Plasma samples are fractioned using size exclusion chromatography, then analyzed with gel electrophoresis and differential scanning calorimetry (DSC). DSC thermograms of plasma fractions are compared against controls to show diagnostic trends.

Results: Thermograms of fraction 11 show increased heat capacity of transitions centered at 50, 62.5, and 95 C. By comparing to standard pure protein thermograms and gels, it is inferred that fibrinogen, IgA, IgG, alpha-2-macroglobulin, and haptoglobin are possibly elevated or experiencing novel protein interactions. Fraction 20 shows increased heat capacity of the 62.5, 75, and 95 C peaks, indicative of C-reactive protein, complement C3, albumin, fibrinogen, ceruloplasmin, IgG, and prealbumin involvement. Fraction 27 shows increased heat capacity of the 62.5, 77.5, and 92.5 C peaks, indicative of albumin, immunoglobulin, and complement C4 involvement.

Conclusions: The results, while all different than the control, are highly variable with a few themes—consistent elevation of haptoglobin, fibrinogen, and immunoglobulins. Plasma fractions are currently being analyzed by mass spectrometry to discern the nature of modifications within the ovarian cancer proteome. The eventual goal of this project is to explore the differences in plasma proteomes between ovarian cancers of various origins.

Funding provided by National Cancer Institute grant R25-CA134283

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3700

Close Window

Title: Inhibition of melanoma metastases by targeting regulator of G protein signaling 2 (RGS2) [2014]

Authors: Deepa Patel,[1] Deyi Xiao,[1] Hongying Hao,[1] Kelly M McMasters.[1] Surgery.[1]

Keywords: melanoma, G protein signaling, EMT, MAPK

Abstract:

While new targeted therapies for melanoma have been developed recently, the prognosis for metastatic melanoma remains dismal. To that end, the investigation of mechanisms for melanoma progression is an important translational research area that can bridge basic science with clinical practice. In cutaneous melanoma, the mitogen activated protein kinase (MAPK) is constitutively activated. Activation of this pathway leads to the induction of epithelial-to-mesenchymal transition (EMT), a process that resembles the genesis of cancer stem-like cells, resulting in tumor invasion, aggressiveness, and metastasis. The regulator of G protein signaling (RGS) protein plays an important role in the development of vasculature. RGS family members are regulatory molecules that act as GTPase activating proteins (GAPs) for G alpha subunits of heterotrimeric G proteins. However, none of the current research has entailed the connection between RGS and melanoma progression. This project used an established melanoma progression model to demonstrate that one of the RGS family members, RGS2, was upregulated in highly metastatic melanoma cell lines. Upregulation of RGS2 in metastatic cell lines was also associated with hyperactivated MAPK pathway and induction of EMT. Manipulated expression of RGS2 caused increased migration and invasion abilities in less-metastatic melanoma cells by RGS2 plasmid transfection or decreased metastatic potential by RGS2 siRNA knockdown in highly-metastatic melanoma cells. These results confirm that over expression of RGS2 can promote melanoma metastasis by inducing EMT through activation of the MAPK pathway. It provides a starting point for mechanism-based evidence in support of RGS2 as a therapeutic target for inhibiting melanoma progression.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3962

Close Window

Title: Isolating miRNAs and their mRNA Targets in Lung Adenocarcinoma Tumors versus Normal Adjacent Lung Tissue [2014]

Authors: David Patterson, Andrea Angstadt, Carolyn Klinge. Biochemistry & Molecular Biology.[1]

Keywords: miRNA, Lung, Adenocarcinoma, Tumor, HITS-CLIP

Abstract:

MicroRNAs (miRNAs) act as oncomiRs, tumor suppressors, and as post-transcriptional regulators of target mRNAs by binding to sites in the mRNA 3'UTR in the RNA-induced silencing complex (RISC) containing Argonaute (Ago) proteins. Published studies of miRNA in lung cancer provide a global level of expression and may not be indicative of miRNA functionality, which is important for the identification of candidate therapeutic targets. This study hypothesized that miRNAs and mRNAs interact with one another in complex reaction networks that differentiate lung adenocarcinoma, a type of non-small cell lung cancer (NSCLC) that is increasing globally in smokers and non-smokers, from normal adjacent lung tissue. A newly developed protocol, high-throughput sequencing together with UV-crosslinking and immunoprecipitation (HITS-CLIP), was performed on A549 cells and flash frozen lung adenocarcinomas and normal adjacent tissue. This study uniquely isolated miRNA-mRNA complexes by immunoprecipitation with the Ago2 protein using radiolabeled linkers to follow the complex during purification. Further RNA isolation was performed by protease digestion and phenol-chloroform extraction. RT-PCR and subsequent gel extraction was utilized to amplify bound miRNA-mRNA complexes to which linkers were ligated and the tagged RNA has been sent for RNA sequencing. This study successfully isolated and prepared for sequencing stable miRNA-mRNA complexes bound to Ago2 in lung adenocarcinomas and normal adjacent tissues. Once the samples are sequenced, this study will enter the bioinformatics stage to identify the differentially expressed miRNA-mRNA complexes in lung adenocarcinoma versus normal adjacent tissue. Funding was provided by NCI R25-CA-134283 and a Kentucky Lung Cancer Research Program grant.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3939

Close Window

Title: Development of Theranostic Mesoporous Silica Nanoparticles for Pancreatic Cancer [2014]

Authors: Dillon Pender,[1] Anil Khanal,[1] Shanice Hudson,[1] Lacey McNally.[1] Medicine.[1]

Keywords: Pancreatic Cancer, Theranostic, Nanoparticle, Silica

Abstract:

Introduction: Modern methods of pancreatic cancer diagnosis and treatment are severely lacking and have failed to provide effectual treatment options for patients. A potential solution for tumor-targeting difficulties is through the implementation of nanotechnology. We hypothesize that pH-responsive chitosan-capped mesoporous silica nanoparticles (MSNs) with the targeting ligand, urokinase plasminogen activator (UPA) will serve as theranostic agents for treatment and diagnosis of pancreatic cancer.

Methods: MSNs were synthesized by employing cetyl trimethylammonium bromide (CTAB), tetraethyl orthosilicate (TEOS) and chitosan through the sol-gel method. The synthesized MSNs were characterized by transmission electron microscopy (TEM) and zeta-potential measurements. MSNs were tagged with UPA to increase nanoparticle binding efficiency towards pancreatic tumor cells. The binding efficiency of both tagged and non-tagged MSNs were observed at various pHs. Finally, UPA-tagged MSNs were injected into mice infected with tumors cells to observe the distribution of these nanoparticles *in*-vivo by multispectral photoacoustic Tomography system (MSOT).

Results: TEM pictures showed the synthesized MSN had a size around 120 nm. Zeta-potential measurements revealed that charge density of MSN dependent on pH. Fluorescence microscopy, Odyssey infrared imaging and tissue phantoms showed that uptake of MSNs by pancreatic tumor cells depended on the pH and tagging of UPA. Lowering a pH and tagging a ligand drastically increased the uptake of MSNs in pancreatic tumor cell *in vitro*. Furthermore, UPA loaded MSNs were successfully detected in orthotopic pancreatic tumor of mice within 6 hours of imaging time by MSOT.

Conclusion: UPA tagged, pH sensitive MSNs demonstrate potential as a theranostic nanoparticle for pancreatic cancer.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3701

Close Window

Title: Developing an Immunocompetent Mouse Lung Cancer Model for the Evaluation of Virotherapy Effectiveness [2014]

Authors: Eric Riedinger,[1] Jonathan Nitz,[1] Kelly M. McMasters,[2] Jorge G. Gomez-Gutierrez.[1] The Hiram C. Polk MD Department of Surgery[1] and The Hiram C. Polk MD Department of Surgery and James Graham Brown Cancer Center.[2]

Keywords: oncolytic, adenovirus, immunocompetent, mouse, lung, cance, model

Abstract:

The preclinical characterization of oncolytic adenoviral vectors has so far been restricted to immunodeficient xenograft tumor models because human adenoviruses do not replicate efficiently in murine tumor cells. While these immunodeficient animal models demonstrate effective replication in and destruction of human tumors by adenovirus type 5 (Ad5)-based vectors, the viruses do not replicate in mouse tissues and the models thus cannot assess the complete safety and efficacy profile of the vectors in normal tissue, nor do they permit evaluation of the impact of an active immune system on overall vector potency. In contrast, the effect of virus replication and the immune response could be evaluated in an immunocompetent syngeneic tumor model. Previously the chemotherapeutic agent Temozolomide (TMZ) was used to enhance virotherapy effectiveness in human glioblastoma cells. We hypothesize that TMZ will enhance oncolytic Ad replication in mouse Lewis Lung Carcinoma-1 (LLC-1) thereby inducing a potent cancer cell killing effect. This study evaluated the ability of TMZ to enhance oncolytic Ad replication in the syngeneic mouse C57BL LLC-1 cell line.

LLC-1 cell line was infected with an oncolytic Ad (Adhz60-E1B deleted) followed by TMZ treatment, it was found that TMZ increases Ad E1A expression, a key component of Ad replication machinery. The productive replication was further validated via detection of Ad structural late proteins and virus quantification. The combination of Adhz60 with TMZ resulted in a synergistic cancer cell killing effect. This study provides evidence that LLC-1 treated with TMZ became susceptible to oncolytic Ad replication.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3946

Close Window

Title: Predicting adverse events in patients undergoing hepatectomy – validation of preoperative nomogram and risk score. [2014]

Authors: Travis Spaulding,[1] Robert C.G. Martin II, MD, PhD.[1] Department of Surgery, Division of Surgical Oncology.[1]

Keywords: hepatectomy, morbidity, nomogram, preoperative, complication, malignancy, primary, metastatic

Abstract:

Aims: There is much published research on preoperative measures of postoperative mortality in the surgical treatment of liver malignancies, but little on morbidity, a much more common postoperative outcome. *Dhir et al.* and *Simons et al*. have a published preoperative nomogram and perioperative risk score, respectively, assessing the risk of mortality post-hepatectomy. The aims of this present study were two-fold: one, to validate the published calculations as acceptable measures of postoperative mortality and two, to asses the value of these published measures in predicting postoperative morbidity.

Methods: Data was collected from a prospectively managed dataset of 1059 hepatectomies performed in Louisville, Kentucky from December 20, 1990 to April 11, 2014. Preoperative data was used to assign scores for each published measure and the scores were sorted into clinically relevant groups with corresponding ordinal scores, according to the previously published literature.

Results: After selection, 851 hepatectomies were analyzed. Both the *Dhir et al.* nomogram (p=0.0004) and *Simons et al.* risk score (p=0.0017) were acceptable predictors of postoperative mortality. In the analysis of morbidity, scores according to *Dhir et al.* were a poor predictor of morbidity. The ordinal risk score published by *Simons et al*. was predictive of complications (p=0.0029), the number of complications (p=0.0028), complication grade (p=0.0033), and hepatic-specific complications (p=0.0003).

Conclusions: The grouping of perioperative risk scores, according to *Simons et al*., provides physicians and patients with a clinically useful measure of risk of postoperative morbidity after hepatectomy.

Acknowledgements: National Cancer Insititute—Grant R25-CA134283

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3945

Close Window

Title: Targeting cytosolic aspartate aminotransferase in human pancreatic and lung carcinoma using a novel inhibitor in vitro [2014]

Authors: Govind Warrier,[1] Yoannis Imbert-Fernandez,[2] Rob Spaulding,[1] Jordan Noe,[1] Brian Clem,[2] John Trent,[2] Jason Chesney.[2] Medicine[1] and Other.[2]

Keywords: aspartate aminotransferase, metabolism, cancer

Abstract:

Increased glycolytic metabolism is a hallmark of neoplastic cells that allows self-promotion of growth and survival. The malate-aspartate shuttle (MAS) plays a significant role in optimizing energy output from glycolysis by facilitating the transfer of electrons from cytosolic NADH for use in mitochondrial electron transport. A key enzyme in the MAS pathway is aspartate aminotransferase (AAT/GOT1), of which there are cytosolic (cAAT) and mitochondrial (mAAT) variants. Recently, AAT has also been found to be integral in proliferation of human pancreatic ductal adenocarcinoma (PDAC) through its role in increasing the $NADPH/NADP^+$ ratio allowing maintenance of the cellular redox state (2). Studies show inhibition of AAT with aminooxyacetate (AOA), a known inhibitor of transaminases, decreases proliferation of PDAC and breast adenocarcinoma cells (1).

Based on the overexpression of AAT in carcinoma containing Ras oncogene mutations, along with the critical role of AAT in the aforementioned metabolic pathways, we hypothesize that AAT may be a suitable target for future cancer therapeutics. Through an active site binding screen of cAAT, novel inhibitors 117 and 4-47 were discovered. 117 and 4-47 were both found to decrease cell growth in pancreatic adenocarcinoma, alveolar adenocarcinoma, and large cell lung cancer, in tissue culture. Treatment with 4-47, in particular, displayed drastic reductions in growth. An in vitro AAT assay revealed inhibition of cAAT activity by 4-47. These findings demonstrate 4-47 to be an inhibitor of cAAT warranting further investigation as a potential therapeutic.

Research supported by the NIH/NCI R25-CA-134283 grant and the University of Louisville Cancer Education Program.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3929

Close Window

Title: Expression and Analysis of GST-tagged UBR5 Protein [2014]

Authors: Dexter Weeks,[1] Kumar Saurabh,[1] Levi Beverly.[1] Medicine.[1]

Keywords: UBR5, ubiquitination, GST, lung cancer, UBR-box

Abstract:

Introduction: Investigation of ubiquitination and proteasome degradation pathways is essential to the field of cancer biology and therapeutics because of their role in cellular proliferation and apoptosis. Of particular interest is the targeting of UBR5 due to its potential role in solid tumor formation. Our previous data demonstrate that siRNA mediated loss of UBR5 is detrimental to lung cancer cell survival. We hypothesize that knockout of UBR5 provides chemotherapeutic benefit in mouse models for lung cancer.

Methods: The objective of this study was to induce bacterial cultures to express and purify the UBR-box of UBR5 proteins. After harvesting and lysis, our aim was to analyze the lysates for the presence of the desired proteins and solubility, which would be conferred by the recombinant tags. Subsequently, the findings of this process would allow for future investigation into the biochemical mechanisms by which putative inhibitors of UBR5 act. We grew cultures of recombinant E. coli induced to express GST, GST-tagged UBR-box with 35 amino acid of flanking sequences, and GST-tagged UBR-box with 100 amino acid of flanking sequences. Next, each respective E. coli culture was lysed to obtain soluble GST-tagged proteins.

Results: Western blot analysis confirmed expression and solubility of the desired recombinant proteins. The findings revealed the E. coli cultures expressed their desired GST-tagged proteins, however only the GST-only culture produced protein found in the soluble fraction after lysis.

Conclusion: Experimentation on purified UBR5 protein will uncover the benefits of UBR5-specific inhibition as a cytotoxic therapeutic approach for lung cancer.

Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3918