UCE-107

Title: MiRNA Regulators of Cell Survival as Biomarker Predictors of Prostate Cancer [2012]

Authors: April Aloway,[1] Dominique Jones,[1] Divine-Favour Anene,[1] Praise Anene,[1] Christina Hickey,[1] Shirish Barve, Ph.D,[2] Lacey McNally, Ph.D,[2] LaCreis Kidd, Ph.D.[2] Pharmacology[1] and Medicine.[2]

Keywords: Prostate Cancer, MicroRNA(miRNA), apoptosis, Quantitative Real-Time PCR

Abstract:

Prostate Cancer is the second leading source of cancer related deaths among American men. The need to find biomarkers that correlate with metastatic prostate cancer is imperative as metastatic disease has a poor survival rate due to limited prognostic tools. MicroRNA (miRNA)are short, non-coding intrinsic RNA that are shown to regulate cellular events in cancer, such as apoptosis, by interacting with mRNA. This study aims to develop a novel technique to identify miRNA that impact apoptosis in advanced prostate cancer and possibly lead to a new diagnostic technique for metastatic prostate cancer. We propose that in comparison to disease-free men, men diagnosed with prostate cancer will have higher serum levels of miRNA that suppress apoptosis and lower levels of cell death related miRNA. We further postulate a stage-dependent expression pattern exists. Specifically, men with more advanced disease will have greater dysregulation as compared to their indolent diseased counterparts. To conduct this study, Serum (Bioserve Biotechnologies, Ltd) samples were obtained from Caucasian men with 3 distinct disease states: disease free men (n=5), men with stage 1 (n=5), and men with stage 4 (n=5) Prostate Cancer. Total RNA was extracted from samples, reverse transcribed into cDNA. Resultant cDNA was further analyzed using qRT-PCR via Taqman low-density Human miRNA array targeting 384 miRNAs. Statistical analysis of data was executed using Expression Suite Software version 1.0. Potential miRNA effectors of apoptosis were determined by Ingenuity analysis for miRNA that are experimentally shown or have high computational probability to effect pathways involved in apoptosis. Our results show a differential expression pattern of miRNA in serum samples and shows promise in development in cancer screening.

UCE-108

Title: MicroRNA Prostate Cancer Signatures Involved In Cell Adhesion and Angiogenesis [2012]

Authors: Divine-Favour Anene, Biology,[1] Dominique Jones,[2] Christina Hickey,[2] April Aloway,[2] Praise Anene,[3] Lacey McNally,[2] Shirish Barve,[2] LaCreis Kidd.[2] Biology,[1] Pharmacology and Toxicology[2] and Other.[3]

Keywords: MicroRNAs

Abstract:

This project will focus on identifying MicroRNAs (miRNAs) that regulate cell adhesion and angiogenesis which could be used as biomarkers for prostate cancer. We aim to use this information as a reasonable alternative to current prostate cancer (PCa) staging and diagnosis techniques. This possibility arises from the post-transcriptional control exerted by miRNAs on a vast number of body processes as they target messenger RNA (mRNA) transcripts. To analyze this behavior, expression analysis was carried out using Taqman PCR array cards on PCa blood samples (of different PCa stages) gotten from African-American men. This process involved the isolation of total RNA, cDNA amplification, and real-time PCR, with a subsequent analysis of the results obtained (ongoing at the moment). We hope to receive data that shows differential expression of the different miRNAs across varying stages of disease. If successful, the identification of these miRNAs should provide an improved basis for PCa diagnosis and staging as compared with the Prostate Specific Antigen (PSA) test that currently serves as the gold standard for this purpose.

Title: An antibody-based method for the detection of early stage lung cancer [2012]

Authors: James Bradley,[1] Kavitha Yaddanapudi, PhD,[1] Sharon Willer,[1] Jason Chesney, MD, PhD,[1] John Eaton, PhD.[1] Medicine.[1]

Keywords: autoantibody, lung cancer, detection

Abstract:

Lung cancer is the major cause of cancer-related deaths worldwide, accounting for about 1.3 million deaths annually. Approximately $10.3 billion is spent on lung cancer treatment in the United States each year. Often, clinical symptoms only appear during later stages of cancer development, or a diagnosis is made after an x-ray is done for other medical reasons. Various screening strategies have been tested for detection of early stage lung cancer but only one cumbersome technique (low-dose computed tomography) has shown any promise. We have developed an inexpensive and fast alternative blood test that we currently believe will detect antibodies appearing early in the development of lung cancer. The test involves flow cytometric analyses of A549 (human lung adenocarcinoma) cells incubated with dilute patient serum and a secondary anti-human IgG antibody (tagged with the fluorescent tag, R-Phycoerythrin). In very early tests of this system, we found two of four serum samples from stage I non-small cell lung cancer patients were positive for antibodies against these cells. A potential strength of this approach is that it is a broad-spectrum screen that will identify antibodies against a variety of cell surface antigens. In addition to detecting lung cancer in its earliest stages, our technique also offers the exciting possibility of identifying novel human lung cancer antigens that may serve as targets for future therapy.

UCE-109

Title: Gene Expression in Breast Carcinomas from Patients with Ethnical Differences [2012]

Authors: Adrienne Bushau,[1] Sarah Andres, Ph.D.,[1] James Wittliff, Ph.D., M.D. hc.[1] Biochemistry & Molecular Biology.[1]

Keywords: breast cancer, race, gene expression

Abstract:

Background: African American women often exhibit more aggressive breast cancer and have a higher mortality rate than Caucasian women. Differences in cultural and socioeconomic status are possible explanations for the higher mortality rate and more advanced stage of breast cancer. However, numerous studies suggest that differences in insurance coverage and socioeconomic status do not explain the observed differences observed in clinical behavior of breast carcinomas of black and white patients, suggesting a biological basis. The goal was to ascertain if dissimilarities occur in gene expression of breast carcinoma biopsies of white and black patients, and further to evaluate if these gene expression differences were related to cancer behavior.

Materials and Methods: Using an IRB-approved biorepository and database, gene expression levels were compared in tissue biopsies from white and black patients utilizing microarray analyses of LCM-procured breast carcinoma cells and assessed with clinical information. These studies generated a candidate gene list, which included TRAPPC2L, CRYBB2P1 and PDHA1 to be validated by qPCR. Then frozen tissue sections from de-identified patients diagnosed with primary breast carcinoma and metastasis were utilized. Total RNA was extracted from frozen intact tissue sections with the RNeasy® Mini Kit (Qiagen Inc.). Integrity of RNA was analyzed using the Bioanalyzer 2100 (Agilent Technologies). Total RNA was reverse transcribed using iScript (Biorad). Primers were designed using Primer Express (Applied Biosystems) and Primer Blast (NCBI). RNA quantification and analyses were performed in triplicate by qPCR in duplicate wells using the ABI Prism® 7900HT (Applied Biosystems) with Power Sybr® Green (Applied Biosystems) for detection. Relative gene expression was calculated using the ΔΔCt method with cDNA prepared from Universal Human Reference RNA (Stratagene) as both a calibrator and a standard for quantification of RNA using β-actin (ACTB) as a reference gene. T-tests, box and whisker and Kaplan-Meier plots were performed in Graph Pad Prism. Pearson correlations and Cox regressions were performed in SPSS Statistics 20. The gene interactions were evaluated with Ingenuity IPA.

Results: Examination of candidate gene expression levels from microarray analyses revealed that CARD11 ($P=0.001$), TRAPPC2L ($P<0.001$), CRYBB2P1 ($P<0.0001$) and PDHA1 ($P<0.0001$) exhibited significant differences in breast carcinomas of African American patients compared to those of Caucasian patients. Of these genes, only PDHA1 expression was correlated with overall survival ($P=0.05$) when the entire population of 245 breast carcinoma patients was stratified by median gene expression level without regard to race. Only PDHA1 expression assessed by microarray was correlated with overall survival ($P=0.04$) of white patients when stratified by race and gene expression level. From the expression levels of 20 genes found most significant by T-test of the microarray data, PDHA1, CRYBB2 and TRAPPC2L were investigated further by qPCR. Using a platform comparison of gene expression levels of candidate genes from qPCR and microarray, CRYBB2 was significantly correlated ($P=0.01$), while PDHA1 was insignificantly correlated ($P=0.22$). From analyses of tumor marker protein results, significant differences were detected in estrogen and progestin receptor gene expression levels in tissue biopsies when comparing white and black patients and white and hispanic breast carcinoma patients.

Conclusions: Expression levels of CARD11, PDHA1, TRAPPC2L and CRYBB2P1 were significantly different in breast tissue biopsies of African American patients when compared to those of Caucasian patients. Of the four genes, only PDHA1 gene expression levels of LCM-procured carcinoma cells were significant when correlated with overall survival of the entire population ($n=245$) regardless of race. Furthermore, PDHA1 expression was correlated with overall survival when only white patients were considered. Although dissimilarities in gene expression levels were observed in black and white patients, preliminary evaluation of a limited gene subset to personalize prognosis assessment requires additional studies related to a patient's ethnical background.

Supported in by a grant from the R25 National Cancer Institute Summer Research Program, PHS Grant # 1 R25 CA 134283-1 to AMB.

UCE-110

Title: In Silico Screening for Novel Human Arylamine N-Acetyltransferase 1 Inhibitors [2012]

Authors: Samantha M. Carlisle, B.S.,[1] Carmine S. Leggett, Ph.D.,[1] John O. Trent, Ph.D.,[2] Mark A. Doll, M.S.,[1] J. Christopher States, Ph.D.,[1] David W. Hein, Ph.D.[1] Department of Pharmacology and Toxicology[1] and Department of Medicine.[2]

Keywords: NAT1, arylamine, in silico

Abstract:

Human arylamine *N*-acetyltransferase 1 (NAT1) is a phase II xenobiotic-metabolizing enzyme that plays an important role in the deactivation and bioactivation of many environmental carcinogens such as, 4-aminobiphenyl (ABP) which is found in cigarette smoke. Arylamines, such as ABP can either be *N*-acetylated (deactivation) by NAT1, or if first acted upon by a cytochrome P450 enzyme, they can be *O*-acetylated (bioactivated) by NAT1. Once bioactivated, these compounds form arylnitrenium ions leading to DNA adducts. If not repaired by nucleotide excision (NER), these DNA adducts can lead to mutagenesis and cancer initiation. Human NAT1 is therefore an ideal molecular target for cancer therapy. Compound **10**, an effective inhibitor of human NAT1 was previously discovered using *in silico* screening. This compound has been shown to decrease human NAT1 activity, ABP-induced DNA adducts, cell proliferation, and cell invasion in human breast adenocarcinoma cells. We hypothesized that we could identify novel small molecule inhibitors of human NAT1 by building upon the previous *in silico* studies through the utilization of an updated ZINC library and also by performing a similarity search to compound **10**. The two new *in silico* searches performed identified 161 potential NAT1 inhibitors, 35 of which were tested for their ability to inhibit human NAT1 *in vitro*. From this initial screening, compounds **63**, **66**, **72**, **86**, and **95** were chosen as lead compounds because they experimentally inhibited human NAT1 greater than 75% *in vitro*. We determined the IC_{50} values of these lead compounds to be 66.1, 5.74, 161, 100, and 102 µM, respectively. The most potent inhibitor, compound **66**, was chosen for further experiments. Compound **66**, inhibited human NAT1 recombinantly expressed in yeast (*in vitro* IC_{50} = 5.57 µM) and endogenously expressed in human breast cancer cells (*in situ* IC_{50} = 2.44 µM). Compound **66** was tested against human NAT2 (*in vitro* IC_{50} = 12.8 µM) to determine which isozyme of NAT it preferentially binds to, with the results showing a preference for human NAT1. [Partially supported by the University of Louisville Cancer Education Program (NCI R25-CA134283).]

UCE-111

Title: Echinomycin Decreases Cell Viability of Pancreatic Adenocarcinoma Cells through Inhibition of Autophagy [2012]

Authors: Jeffery Farmer,[1] Justin Huang,[1] Lacey McNally, Ph.D.[2] Other[1] and Medical Oncology.[2]

Keywords: Pancreatic Adenocarcinoma, Autophagy, Hypoxia, Echinomycin

Abstract:

Objective: Pancreatic carcinomas are the fourth leading cause of cancer death in the United States. Because pancreatic cancers respond poorly to conventional chemotherapy, investigation of new drug treatment options is needed. We hypothesized that echinomycin, an inhibitor of hypoxia-inducible factor-1 (HIF-1), would significantly decrease cell viability of pancreatic cancer cell lines in a hypoxic environment.

Methods: Highly metastatic pancreatic adenocarcinoma cell lines S2VP10 and S2CP9 and the non-metastatic pancreatic adenocarcinoma cell line MiaPaCa2 were treated with echinomycin in normoxic (20% O2) and hypoxic (5% O2) conditions. Following treatment with 1 nM, 5 nM, 10 nM, 20 nM, and 40 nM echinomycin, cell viability after 24 hours, as a measure of ATP, was compared to an untreated normoxic control in all cell lines. Mechanism of induced cell death by echinomycin was evaluated using cell stress and apoptosis profiling. Proteins from treated cell lines and untreated control cell lines were harvested and analyzed by western blotting to evaluate changes in the autophagy pathway.

Results: In all three cell lines treated with 10 nM of echinomycin, cell viability at twenty-four hours was less than 50% in comparison to the normoxic control. Accumulation of prominent autophagosomes was evident in treated cell lines twelve hours after treatment. Cell stress array revealed a prominent increase in heat shock protein 60 (HSP60) in treated S2VP10 cells in comparison to untreated S2VP10 cells. Although apoptosis array revealed extremely high levels of pro-caspace-3, cleaved caspace-3 did not increase in treated S2VP10 cells. In all treated cell lines, levels of the autophagy marker protein light chain 3-II (LC3-II) were greatly increased in comparison to untreated cell lines.

Conclusion: Our results indicate that echinomycin inhibits cell viability of metastatic pancreatic cancer cells through inhibition of autophagy rather than through apoptosis. Treatment with echinomycin in a hypoxic environment resulted in higher levels of cellular death in comparison to normoxic conditions. Echinomycin has potential to be used in combination drug therapy as a treatment for pancreatic adenocarcinoma.

This work was supported by the R25 Cancer Education program (NIH/NCI grant R25--CA134283) at the University of Louisville.

GPH-100

Title: Examination of combined effects of well-done red meat, smoking, and rapid N-acetyltransferase 1 and 2 on breast cancer susceptibility [2012]

Authors: Christina Hickey, MPH,[1] LaCreis Kidd, MPH/PHD,[2] David Hein, PHD,[2] Mark Doll.[2] Public Health and Information Science[1] and Pharmacology and Toxicology .[2]

Keywords: Breast cancer , N-acetyltransferase 1, N-acetyltransferase 2, smoking, meat derived, CYP1A2

Abstract:

BACKGROUND: Genetic variations in carcinogen metabolism genes may influence susceptibility to environmentally induced cancers. For instance, inheritance of CYP1A2*F and NAT1*10, linked with increased metabolic activation of pro-carcinogens, have been implicated in the etiology of breast cancer in which cigarette- or meat derived carcinogens have implicated in its etiology. Moreover, possession of the NAT2* slow or very slow genotypes, associated with decreased capacity to detoxify cigarette-derived carcinogens may result in an increase in the risk of developing breast cancer. However, published reports on the joint modifying effects among highly variant biotransformation gene (I.e., CYP1A2, NAT1, NAT2) or environmental exposures on breast cancer susceptibility is limited in scope.

To address this concern, the current study evaluated complex interactions among three variant carcinogen metabolism genes, tobacco smoking, and preferences towards meats prepared well done as modifiers of breast cancer. We hypothesize individuals who inherit two or more high-risk carcinogen metabolism genes (CYP1A2*F, NAT1*10, slow NAT2*5, CYP1A2*F) will have an increased risk of developing breast cancer. These effects may be exasperated by exposure to direct or indirect exposure to cigarette or meat-derived carcinogens.

METHODS: To test out hypotheses, we conducted a case-control study consisting of 930 participants of the Iowa Women's Health Study. Genetic alterations detected I germ-line DNA collected from profiles were obtained from 273 breast cancer cases and 657 controls using TaqMan RT-PCR and RFLP-PCR assays. A lifestyle and food frequency questionnaire were used to ascertain direct and indirect exposure to cigarette and meat-derived carcinogens (i.e., preferences toward meats prepared well done), respectively. Main effects were evaluated and currently MDR is underway.

RESULTS: We observed subjects possessing NAT1*10 (OR, 1.50; 95%CI, 1.07-2.12) or NAT1*11 (OR, 2.08; 95%CI, 0.99-4.36) exhibited significantly higher breast cancer risk. Possessing rapid or intermediate NAT2 acetylator genotypes, breast cancer risk increased significantly (P trend =0.007) with degree of meat doneness. No main effect was observed between CYP1A2 genotype and breast cancer risk and no combined effect of CYP1A2 genotype was observed on the associations NAT2 genotype on breast cancer risk. However NAT1 alleles with CYP1A2 slow/intermediate genotype possessed a 2.43-10.54 fold increase in breast cancer susceptibility. The inheritance of CYP1A2 rapid acetylator genotype show a 1.91-2.74 fold increase in breast cancer risk. There was a combined effect of NAT1 well done meat intake observed in NAT1*3 (OR, 1.82; 95% CI, 1.10-3.00) or NAT1*4 genotype (OR, 1.82; CI, 1.10-3.00). For subjects with NAT1*10 and/or NAT1*11 there was a significant increase in breast cancer in women who consumed well/very well meat (OR, 2.61; CI, 1.52-4.48.

CONCLUSION: The results provide further support for modifying effects of NAT1 and NAT2 genetic polymorphism in breast cancer risk related to aromatic and/or heterocyclic amine carcinogens. This approach will aid in future studies that analyze gene-gene/gene environment interactions to detect increased risk of BrCa in women. [Partially supported by the University of Louisville Cancer Education Program (NCI R25-CA134283)].

UCE-112

Title: UBQLN1 regulates IGF1R signaling pathway in non-small cell lung cancer cells [2012]

Authors: Gretchen E. Holz, B.A.,[1] Levi J. Beverly, Ph.D.[2] Other[1] and Department of Medicine.[2]

Keywords: UBQLN1, IGF1R, cancer

Abstract:

Many growth factor receptors are known to play key roles in the development of cancer, so it is critical to understand the components and mechanisms involved in their signaling pathways. However, current knowledge on these details is limited. The type I insulin-like growth factor receptor (IGF1R) stimulates a signaling pathway that leads to cell growth and proliferation in normal cells, and the IGF1R pathway is often deregulated in human lung adenocarcinoma. Details regarding early players and events in this pathway are incomplete. Our previous data show that Ubiquilin1 (UBQLN1), a cytosolic protein known to be involved in a variety of homeostatic cellular processes, like regulation of protein degradation and receptor trafficking, is upregulated in non-small cell lung cancer (NSCLC) cells. In addition, upregulation of UBQLN1 proves to be predictive of long-term outcome of patients. Herein, we demonstrate a novel protein interaction between IGF1R and UBQLN1, which thus implies UBQLN1 plays a role in regulating the IGF1R pathway. This preliminary research may lead us to a more complete understanding of IGF1R's endocytic pathway, including the mechanisms and components necessary to continue the signal and/or recycle the receptor. Ultimately, these details are of great importance as more than 100 clinical trials have been conducted to assess the potential of inhibiting IGF1R signaling as a cancer therapy. The trials have varied in success suggesting a critical need to target different and more specific avenues for more consistent therapeutic results. Our work hopes to identify novel mechanisms that regulate IGF1R signaling in cancer leading to more effective treatment strategies.

Special thanks for funding provided by NCI R25 grant support University of Louisville Cancer Education Program NIH/NCI (R25- CA134283) and the Kosair Pediatric Cancer Program.

UCE-113

Title: Inhibition of Human Arylamine N-Acetyltransferase I using Curcumin and Resveratrol Increases the Potency of Small Inhibitor Compound 10 [2012]

Authors: Nicole Jackson,[1] Carmine S. Leggett, Ph.D.,[1] Mark A. Doll, M.S.,[1] J. Christopher States, Ph.D.,[1] David W. Hein, Ph.D.[1] Department of Pharmacology and Toxicology.[1]

Keywords: Curcumin, Resveratrol

Abstract:

Human arylamine *N*-acetyltransferase 1 (NAT1) is a phase II metabolizing enzyme that plays an important role in metabolizing environmental carcinogens and cancer progression. NAT1 catalyzes *N*-acetylation of arylamines, as well as the *O*-acetylation of *N*-hydroxylated arylamines. *O*-acetylation leads to the formation of arylnitrenium ions that bind to DNA nucleotides, forming adducts. If unrepaired, these adducts lead to mutagenesis, and can initiate cancer. Previous studies suggest that NAT1 is an important factor in cell adhesion and cell invasion. Studies completed in our laboratory have determined that a small molecule inhibitor, Compound 10, inhibits NAT1 activity, and causes a decrease in cell invasion and cell proliferation in human breast adenocarcinoma cells. In the present study, we hypothesize that Compound 10 is a more effective and/or potent NAT1 inhibitor in combination with chemopreventive agents such as curcumin or resveratrol. We also postulate that the inhibition of human NAT1 by Compound 10 in combination with these chemopreventive agents will further decrease NAT1 metabolism, cell invasion, and metastasis in human breast adenocarcinoma cells. In yeast lysate that recombinantly express human NAT1, Compound 10 at it's IC_{50} of 0.75 μM exhibited 77% NAT1 inhibition, whereas Compound 10 in combination with curcumin (100 μM) exhibited 90% inhibition ($p < 0.001$) and in combination with resveratrol exhibited 97% inhibition ($p < 0.001$). Compound 10 also dramatically increased the potency of curcumin and resveratrol as inhibitors of human NAT1 recombinantly expressed in yeast. The IC_{50} for curcumin decreased over 10,000-fold, from 134 to 0.0131 μM in the presence of Compound 10 at its IC_{50} of 0.75 μM. Similarly, the IC_{50} for resveratrol decreased over 700-fold, from 1230 to 1.73 μM in the presence of compound 10 at its IC_{50} of 0.75 μM. In MDA-MB-231 human breast adenocarcinoma cells, Compound 10 exhibited 68% NAT1 inhibition in situ whereas Compound 10 in combination with curcumin exhibited 91% inhibition ($p<0.001$) and Compound 10 in combination with both curcumin and resveratrol exhibited 95% inhibition ($p <0.001$). A combination of 100 μM Compound 10 with 100 μM curcumin slightly but significantly ($p<0.05$) reduced cell invasion compared to treatment with Compound 10 or curcumin alone. Similarly, cell adhesion was reduced significantly more by the combination of Compound 10 and curcumin ($p<0.05$) as well as the combination of Compound 10 and resveratrol ($p<0.05$), compared to Compound 10 alone. In conclusion, Compound 10 was synergistic with other chemopreventative agents in human NAT1 inhibition, cell invasion, and cell adhesion assays. Its usefulness in combination with these agents should be further explored as a cancer treatment option. [Partially supported by the University of Louisville Cancer Education Program (NCI R25-CA134283).]

UCE-115

Title: Computational Tools for the Identification of Detectable Uncharacterized Derivatized Metabolites within the Context of Known Metabolic Networks [2012]

Authors: Joshua Mitchell, B.S.,[1] Hunter Moseley, PhD.[1] Chemistry.[1]

Keywords: Metabolomics, Mass Spectrometry, Informatics, Metabolism

Abstract:

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Metabolism is the set of chemical reactions that occur in living systems that make life possible. The majority of these chemical reactions are catalyzed by protein enzymes which interconvert a vast network of organic molecules (metabolites) into forms needed for cells to live and grow. Detection and identification of these metabolites is key to modeling and understanding these large and intricate metabolic networks. Advances in metabolomics, especially in ultra-high resolution mass spectrometry enable the rapid analysis of tens of thousands of peaks (observables) representing a few thousand metabolites. However, a key barrier to meaningful interpretation of this mass spectrometry (MS) data is the identification of detectable but unknown metabolites from mass spectrometry. Recent development of chemoselective (CS) probes that tag metabolite functional groups not only boosts the speed and accuracy of metabolite detection, but provides new computational avenues for metabolite identification. We have developed algorithms that combine this additional functional group information with molecular formulas to improve metabolite identification. These algorithms enable combined molecular formula and functional group searches of metabolite and organic compound databases via fast detection of functional groups in molecular compound databases. This ability to identify the functional groups and the molecular formulas of query structures lends itself to the identification of compounds via chemoselective tagging and/or adduct formation with detection in ultra-high resolution mass spectrometers like the Fourier transform-ion cyclotron resonance-MS (FT-ICR-MS). Furthermore, we have created SQLite databases containing this functional group information for databases such as the Human Metabolome Database (HMDB) and KEGG Compound for use with other computational tools. Using these tools along with chemical adduct information obtained from MS data, exact matches can be found for some uncharacterized metabolites and similar molecules can be found when exact matches cannot be found. Correlations between similar compounds can help generate hypotheses for where newly discovered metabolites fit within metabolic networks of interest like human metabolism. Furthermore, using these tools, the distribution of isomers within and between databases can also be determined. This systematic analysis of metabolite databases will both determine the limitations of these combined analytical and computational approaches and provide clear direction for improvement of these methods.

UCE-116

Title: Candidate Drugs Target the APC/C to Induce Mitotic Arrest in Ovarian Cancer [2012]

Authors: Douglas J. Saforo,[1] Brian C. Sils, B.S.,[2] B. Frazier Taylor, MD/PhD,[2] John O. Trent, PhD,[2] J. Christopher States, PhD.[2] Pharmacology and Toxicology [1] and Pharmacology and Toxicology.[2]

Keywords: Anaphase Promoting Complex, Mitotic arrest, Drug Targeting, Ovarian cancer, Apoptosis

Abstract:

Taxanes are a class of chemotherapeutic drug that act to disrupt microtubule function and cause mitotic arrest and cell death. These drugs are commonly used for cancer treatment, however their effectiveness is dependent on the presence of a functioning spindle assembly checkpoint (SAC). As a result, it is possible for cancer cells to become resistant to microtubule disrupting drugs by inactivating the SAC. The anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase that acts as the master regulator of cell cycle progression. Inhibition of the APC/C should result in disruption of the cell cycle, resulting in arrest during mitosis and/or pseudo-G1. Previous *in silico* studies of the APC/C structure have yielded potential compounds that bind to the APC/C subunit ANAPC2. Three of these compounds (#8, 10, 11) were tested on A2780/CP70 and SKOV3 ovarian carcinoma cells and tGM24 telomerase immortalized human fibroblasts. Cell morphology was observed during treatment with the compounds and showed signs of mitotic and apoptotic cells in a dose dependent manner. While all compounds showed the predicted effects, effects were more pronounced with compounds 10 and 11 than compound 8. Mitotic index determinations revealed a significant mitotic delay in both cancer cells and fibroblasts treated with compounds 8, 10, and 11. Compounds 10 and 11 were more potent than compound 8 in inhibition of colony forming ability for all three cell lines. Fibroblasts showed some resistance to compound 8, however fibroblasts exposed to compound 11 showed complete inhibition of cell growth without characteristic morphological signs of apoptosis. All three compounds induced apoptosis in ovarian cancer cells, as indicated by increased caspase 3 activity, but not in fibroblasts. These results indicate that compounds targeting the APC/C can induce mitotic arrest and kill ovarian cancer cells while sparing normal cells. This research was supported by the University of Louisville Cancer Education Program NIH/NCI grant R25-CA134283.

UCE-117

Title: LDH-A as a Potential Therapeutic Target for Non-small Cell Lung Cancer [2012]

Authors: Sabrina Schatzman,[1] Pankaj Seth, PhD,[2] Pawel Lorkiewicz, PhD,[3] Katherine Sellers, MS,[3] Teresa Fan, PhD.[3] Chemistry,[1] Other[2] and Arts & Sciences.[3]

Keywords: Cancer Metabolism, TCA Cycle, Lactate Dehydrogenase, Stable Isotope Tracers, NMR, MS

Abstract:

Lung cancer is the leading cause of cancer related deaths in both men and women. Despite this alarming statistic, targeted therapies remain elusive. For this reason, there is a need to study cancer cell metabolism in order to find novel therapeutic approaches. Lactate dehydrogenase (LDH) catalyzes the interconversion of pyruvate and lactate. Suppression of LDH-A, a monomor of tetrameric LDH, is thought to cause ROS-mediated apoptosis of non-small cell lung cancer (NSCLC) cells (1). We have characterized the metabolic effects of LDH-A knockdown (KD) in lung cancer metabolism with the use of shRNA in A549 NSCLC cells to gain mechanistic insight into this detrimental effect. $^{13}C_6$-Glucose and $^{13}C_5$-$^{15}N_2$-Glutamine were used as tracers to probe the perturbation of metabolic pathways induced by cells transduced with an anti LDH-A shRNA plasmid. The fate of the carbon and nitrogen isotopes through metabolic pathways was monitored by various analytical techniques including NMR, GC-MS, and FT-ICR-MS. LDH-A KD enhanced the concentration and enrichment of TCA cycle metabolites such as $^{13}C_2$ isotopologues of malate and fumarate when cells were given labeled glucose, supporting enhanced TCA cycle activity which can lead to increased oxidative phosphorylation. The fate of ^{13}C derived from glutamine suggests increased anaplerosis to the TCA cycle from glutaminase. The increased TCA cycle activity as a result of LDH-A knockdown may account for the previously observed increase in ROS production which, in turn, leads to cell death (1).

This work was supported by the University of Louisville Cancer Education Program NIH/NCI (R25- CA134283).

1.Seth P, Grant A, Tang J, Vinogradov E, Wang X, Lenkinski R, Sukhatme VP. On-target inhibition of tumor fermentative glycolysis as visualized by hyperpolarized pyruvate. Neoplasia 2011;13(1):60-71.

UCE-118

Title: Sustained delivery of chemopreventives by biodegradable polymeric implants [2012]

Authors: R. Jennifer Siow,[1] Farrukh Aqil,[2] Radha Munagala,[2] Ramesh Gupta.[2] Arts & Sciences[1] and Pharmacology & Toxicology.[2]

Keywords: polycaprolactone, low molecular weight, polymeric implant, drug delivery, withaferin A, anthocyanidins, curcumin, lung tumor xenograft

Abstract:

Poor oral bioavailability is a major obstacle to the use of natural chemopreventives in the prevention and treatment of cancer. The use of subcutaneous polymeric implants bypasses the oral route and delivers these compounds systemically. Development of multi-layer "coated" implants has further improved this delivery system, reducing the initial burst and providing a more consistent drug release over time. In an attempt to furnish more sustained release, reduce implant degradation time and increase drug release, polycaprolactone (PCL) in four lower-molecular weights (mol wt. 80K, 31K, 18K and 13K Da) were used to formulate coated implants with 10% curcumin as a test agent. The implants were prepared as described (Aqil et al., Cancer Letters, Epub 2012). *In vitro* release of curcumin was measured daily by spectrophotometry over 21 days and cumulative drug release was calculated. Daily *in vitro* release varied with the mol. wt. of the implants. Cumulative release of curcumin from the 80K and 31K implants was found to be similar (~38%) and significantly higher than the release from the 18K and 13K implants (25% and 29%, respectively). Degradation rates were also monitored visually. No change was evident for the first 7-10 days but a slight degradation in 13K and 18K implants was observed over the remaining days.

Three of these polymers (31K, 18K and 13K) were mixed 1:1 (w/w) with PCL-3.6K to determine if the addition of an even lower mol. wt. polymer would facilitate the degradation rate and provide larger drug release. The degradation of the combination implants was observed *in vitro* and *in vivo* using a mouse model for 9 days. Interestingly, 18K:3.6K PCL showed fast degradation resulting in dramatic high release (86%) *in vitro* in 9 days. However, only minor erosion of the implants was observed *in vivo* in the same period of time. The other two implant combinations released almost the same amount as released from the individual PCL implants alone (22-25%).

We have previously demonstrated that anthocyanidins (colored compounds isolated from bilberry) and withaferin A (a terpenoid from the Ayurvedic herb "ashwagandha") inhibit lung cancer in a mouse tumor xenograft model. To analyze the possible synergism between these chemopreventives, athymic nude mice were inoculated with human lung cancer (A549) cells. Mice were then treated with: vehicle or anthocyanidins delivered intraperitoneally (0.5 mg/mouse); withaferin A via polymeric implants (5% w/w); or a combination of both treatments. Consistent with our previous findings, the individual treatments with anthocyanidins or withaferin A inhibited tumor growth by 51% and 53% respectively. The combination treatment showed only slightly increased inhibition of tumor growth (57%). No adverse reactions to the combination treatment were observed. Further testing at different doses is required to determine if anthocyanidins increase the effectiveness of sub-therapeutic doses of withaferin A. (Supported from R25-CA134283 and Agnes Brown Duggan Endowment).

UCE-119

Title: Cancer Stem Cell induced Heterogeneity in Hepatocellular Cancer [2012]

Authors: Vanessa States,[1] Yan Li, M.D./PhD,[2] Su Ping Li, B.S.,[2] Robert Martin, M.D./PhD[2] Arts & Sciences[1] and Surgery- division of surgical oncology.[2]

Keywords: Hepatocellular Carcinoma, cancer stem cell, EpCAM, CD326, chemoresistance

Abstract:

Introduction: Hepatocellular Carcinoma (HCC), is the most common form of liver cancer, and is currently the third leading cause of cancer death worldwide. Current therapies consist of liver resection, transplantation, hepatic arterial drug eluting bead therapy, and/or chemotherapy. The goal of drug eluting bead therapy and chemotherapy is an attempt to target rapidly dividing cells. Initial response rates (at 1-3 months) in HCC is encouraging with either stable disease or partial response. However in most patients the response rates are short and disease progression quickly follows. A small, chemoresistant, subpopulation of stem-like cancer cells are known as cancer stem cells (CSCs.) Like normal stem cells, these CSCs are capable of self-renewal and differentiation, and play a key role in therapy failure and tumor relapse. Understanding the mechanisms of CSCs is imperative for the development of effective therapies. We **hypothesized** that cancer cells are capable of de-differentiating into CSCs under stressful conditions and thus lead to chemoresistance and progression of disease.

Methods: Hepa1-6 (murine HCC) cells were treated with serum free media to induce cancer sphere formation. Cancer spheres were created because they are thought to contain higher percentages of CSCs than cells grown in normal culture conditions. Flow cytometry was used to detect CSC surface markers EpCAM, CD90, CD44 and CD133 in cancer cell spheres. Western blot was used to confirm flow cytometry data as well as to measure key components of the Wnt/β-catenin signaling pathway after treated with 72 hr of doxorubicin. A nude mouse hind limb tumor model was also used to measure the proliferation rate of sphere forming HCC cells.

Results: Flow cytometry data indicate that sphere forming cells contain higher amounts of CSC markers than Hepa1-6 cells grown in normal culture conditions. Median EpCAM expression was increased by 2.0 fold, median CD90 expression was increased by 11.0 fold, median CD44 expression was increased by 1.4, fold and median CD133 expression was increased by 3.0 fold. The western blot data also showed an increased expression of EpCAM in sphere forming cells. These results suggest that HCC cells are capable of de-differentiating into CSCs. In addition, the data show that this mechanism could potentially be related to the over-activation of the Wnt/β-catenin signaling pathway. The nude mouse model indicated that sphere forming cells demonstrated a significantly higher tumor proliferation rate in relation to normal HCC.

Conclusion: These results suggest that HCC cells are capable of de-differentiating into CSCs. In addition, the data show that this mechanism could potentially be related to the over-activation of the Wnt/β-catenin signaling pathway. Understanding of the cellular and molecular events of CSCs in the tumor microenvironment is critical for cancer prevention and therapy.