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Title: Hepatocarcinogenic Effects of 4,4 methylenedianaline (MDA) and Obesogenic Dietary Components. [2014]

Authors: Aditya Barve,[1] Marcus Stepp,[1] Mark Doll,[1] Jingwen Zhang,[2] Gavin Arteel,[1] Shirish Barve,[3] David Hein.[1] Pharmacology/Toxicology,[1] Medicine[2] and Medicine, Pharmacology/Toxicology.[3]

Keywords: environmental carcinogen, obesity, liver , cancer

Abstract:

The rapid progression and high mortality of hepatocellular carcinoma (HCC) make it the 3rd leading cause of cancer death. Historically low incidence rates, particularly in the US, have experienced a steady increase in the occurrence of HCC. In the US 15-50% of HCC patients had no exposure to risk factors like, viral co-infection. This highlights the role of predisposing factors, like diet or exposure to occupational/environmental carcinogens as being critical factors in HCC development. Obesity has been experimentally and epidemiologically linked to increased HCC incidence. Therefore the goal of our study was to investigate the carcinogenic potential of an environmental carcinogen 4,4 methylenedianaline (MDA) singly, and with obesogenic diets.

Male F344 rats were fed either a - normal diet, a high fructose diet (30% in water), or a high fat diet (41 % fat calories) with and without exposure to MDA. Those animals receiving MDA were administered an oral gavage of 10 mg/kg each week for eight weeks. Livers obtained from these rats were subjected to immunohistochemical analysis. Specifically, the effects on the hepatic cell cycle regulator cyclin D1 and the detoxification enzyme glutathione S-transferase (GST-P) were examined, as induction of these proteins are established as reliable markers for hepatic neoplasia. The markers were observed to be increased in response to MDA alone. Importantly, a drastic increase in these markers was observed as a consequence of the combinatorial effect of MDA and diet. Overall, our findings suggest that carcinogenic potential of environmental/occupational carcinogens could be significantly enhanced by diet.

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Title: Combination of Withaferin A and Cisplatin Eliminates Ovarian Cancer Stem Cells [2014]

Authors: Addison Bray,[1] Sham Kakar.[2] Arts & Sciences[1] and Physiology and Biophysics.[2]

Keywords: witheferin A, cisplatin, ovarian cancer, cancer stem cells, ALDH1

Abstract:

Current first round chemotherapy treatments in patients with ovarian cancer often includes a platinum based drug called cisplatin. Cisplatin based treatment is often ineffective and 70% of women will relapse with platinum resistant cancer. The purpose of the present experiments is to understand the effects that cisplatin and withaferin A have on cancer stem cells in ovarian cancer by using aldehyde dehydrogenase 1 (ALDH1) as a biomarker. The expression levels of ALDH1 were tested in vivo by using immunohistochemistry and western blot of mouse ovary cancer tissues after drug treatment. In vitro analysis includes western blot, ALDEFLOUR flow cytometry, and sphere forming assay of $ALDH^+$ and $ALDH^-$ sorted cells. In vivo results suggest that cisplatin raises ALDH1 expression levels, withaferin A lowers ALDH1 levels and a combination therapy lowers ALDH1 levels. This suggests that cisplatin increases the number of cancer stem cells, which explains the high probability of secondary platinum resistant cancer in patients. In vitro results show that both cisplatin and withaferin A reduce ALDH1 expression and act synergistically in combination therapy. Cisplatin treatment may not have enough time to act on the cancer stem cells in vitro or the microenvironment could affect expression. The sphere forming assay shows that only ALDH+ cells are able to form spheres on ultra-low attachment plates in stem cell media. In conclusion, we have shown that ALDH1 expression is up regulated with cisplatin and down regulated with withaferin A or combination treatment. Research supported by NIH/NCI R25-CA-134283.

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Title: A Proposed Treatment Algorithm for Locally Advanced Unresectable Pancreatic Adenocarcinoma [2014]

Authors: Thomas Brenzel,[1] Robert Martin.[2] Arts & Sciences[1] and Surgical Oncology.[2]

Keywords: Pancreatic Cancer, Pancreatic Adenocarcinoma, Unresectable, Locally Advanced, Treatment Algorithm, Stage III

Abstract:

We argue there is currently no standardized staging or treatment algorithm for unresectable locally advanced pancreatic adenocarcinoma. The current NCCN guidelines are vague in the treatment of this disease. The goal of this study was to evaluate current treatments and then propose an acceptable treatment strategy.

Methods: A literature search of major databases was conducted including only articles that focused on treatment and outcomes of specifically stage 3 pancreatic adenocarcinoma.

Results: No clear algorithm exists within the literature. The optimal chemotherapy agent has not been determined while the role and timing of chemoradiotherapy is also disputed. Surgical resection is the optimal treatment for patients who are able to become eligible. We propose patients be treated with induction FOLFIRINOX chemotherapy for 4-6 cycles. Following a reevaluation, patients with stable disease or response should then recieve chemoradiotherapy while patients who progress should continue chemotherapy.

Discussion: FOLFIRINOX is a newer drug combination that has been reported to have increased control in metastatic patients, which we recommend in stage 3 patients initially for the control of metastases. Induction chemotherapy allows for better selection of which patients would benefit from chemoradiotherapy, which has higher toxicity. The goal of treatment is to see a reponse in patients allowing surgical resection. This unresectable stage should be diagnosed by a triphasic CT and laparoscopy to rule out radiologically occult metastatic disease.

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Title: Mechanistic Insight Into Vinyl Chloride-Induced Liver Injury. [2014]

Authors: Adrienne Bushau,[1] Lisanne Anders,[1] Amanda Douglas,[1] Lauren Poole,[1] Veronica Massey,[1] Anna Lang,[1] Cameron Falkner,[2] Matt Cave,[2] Craig McClain,[2] Juliane Beier.[1]
Pharmacology and Toxicology[1] and Medicine.[2]

Keywords: Vinyl Chloride, ER stress, Liver Damage, Chloroethanol, Chloroacetaldehyde

Abstract:

Vinyl chloride (VC), an ubiquitous environmental contaminant, ranks 4th on the ATSDR Hazardous Substances Priority List. It has been shown to cause liver cancer and other hepatic dysfunctions. This study aims to investigate hepatic injury *in vivo* and its mechanisms including the role of endoplasmic reticulum (ER) stress *in vitro*.

Chow-fed C57Bl/6J mice received chloroethanol (ClEtOH), a VC metabolite, and lipopolysaccharide (LPS) after ClEtOH. High fat diet (HFD)-fed mice received a bolus dose of ClEtOH after 10 weeks, 24 hours prior to sacrifice. Liver damage, inflammation, ER stress and changes in carbohydrate and lipid metabolism were determined. HepG2 cells were treated with chloroacetaldehyde (CLA), VC metabolite. RNA was extracted for analysis of ER stress markers. In chow-fed mice, ClEtOH caused no liver damage but caused changes in carbohydrate and lipid regulating genes. LPS exposure caused oxidative stress, lipid accumulation and inflammation, which was exacerbated by ClEtOH preexposure. ClEtOH increased monocyte and neutrophil activation, transaminase levels, necroinflammatory foci and fatty acids.

The combination of ClEtOH and LPS decreased TUNEL-positive cells, suggesting a switch to necrosis. In HFD-fed mice, ClEtOH increased HFD-induced injury, steatosis, infiltrating inflammatory cells, hepatic expression of proinflammatory cytokines and ER stress. CLA decreased oxygen consumption, ATP levels, and mitochondrial function.

Conclusions. VC metabolites sensitize the liver to a "second-hit." This serves as proof-of-concept that VC hepatotoxicity may be modified by underlying liver diseases, which commonly occurs in liver disease. These data implicate VC exposure as a risk factor in the development of liver disease in susceptible populations.

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Title: Development of a shRNA library for high-throughput screening of the role of sphingolipids in tumorigenesis. [2014]

Authors: Cameron Conway,[1] Gauri Patwardhan,[2] Douglas saforo,[2] Levi Beverly,[2] Leah Siskind.[2] Bioengineering[1] and Pharmacology and Toxicology.[2]

Keywords: sphingolipid, shRNA, lung cancer

Abstract:

We seek to develop a comprehensive and unbiased resource that will allow us to study the role of sphingolipid metabolism in lung cancer biology. We are creating a library of viral vectors that will facilitate knockdown of every protein involved in sphingolipid metabolism. This library will be utilized in cellular models to identify sphingolipid genes involved in response of lung cancer to standard of care chemotherapeutics and will allow the scientific community to interrogate the entire sphingolipid metabolic pathway in an unbiased and comprehensive manner. This will increase our understanding of the biological processes regulated by sphingolipids and may lead to the identification of novel therapeutic targets.

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Title: The Hepatic "Matrisome" Responds Dynamically to Stress: Novel Characterization of the ECM Proteome [2014]

Authors: Christine E. Dolin,[1] Veronica L. Massey,[1] Lauren G. Poole,[1] Deanna L. Siow,[1] Michael L. Merchant,[2] Daniel W. Wilkey,[2] Gavin E. Arteel.[1] Department of Pharmacology and Toxicology[1] and Department of Medicine.[2]

Keywords: alcohol, inflammation, extracellular matrix, proteomics

Abstract:

Background. There are no therapies to halt or reverse chronic liver disease (e.g., ALD), which follows a common natural history leading to end-stage liver disease and hepatocellular carcinoma (HCC). Outside the context of fibrosis, the nature and impact of the dynamic responses of the hepatic ECM proteome (i.e., matrisome) to stress are poorly understood. The goal of this work was to develop a proteomic method to characterize the hepatic matrisome and compare the impact of ethanol and lipopolysaccharide (LPS) on this compartment. Methods. Mice were fed ethanol-containing or isocaloric control diet for 6 weeks and injected with LPS or a vehicle 24 hours prior to sacrifice. Liver sections were processed in a series of increasingly rigorous extraction buffers to separate proteins by age and crosslinking. Extracted proteins were identified using liquid chromatography-tandem mass spectrometry (LC-MS/MS). ECM proteins were categorized by primary function and compared between experimental groups. Immunoblotting was performed for fibrin and osteopontin, proteins previously found to be significant in ALD-associated ECM remodeling. Results. The extraction yielded distinct pools of ECM proteins identifiable by LC-MS/MS. The matrisome responded dynamically to stress. Ethanol caused a dramatic ~30% increase in matrisome protein numbers; LPS produced a similar response. The enhancement of LPS-induced liver damage by ethanol preexposure demonstrated unique protein changes. Conclusions. These results suggest that this approach can document qualitative changes to the ECM proteome (i.e., presence and absence). Future work will investigate quantitative matrisome changes. Support: NIH/NCI (5R25CA134283-03) and NIH/NIAAA (1R01AA021978-01).

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Title: Viability of Losartan as a Combination Therapy with Oncolytic Adenovirus [2014]

Authors: Harry Gao,[1] Xiao-Mei Rao,[1] Stephen Wechman,[1] Kelly McMasters,[1] Heshan Zhou.[1]
Medicine.[1]

Keywords: losartan, adenovirus, virotherapy

Abstract:

Cancer-selective oncolytic adenoviruses have great conceptual potential as an emerging cancer therapy. However, one major obstacle in oncolytic virotherapy is maximizing the spread of the virus therapy throughout the tumor. Tumors have an extracellular matrix dense enough to retard or outright halt the distribution of therapeutic nanoparticles like viruses. Combination treatments aimed at degrading or limiting extracellular matrix production hold great promise.

Losartan is a clinically-approved hypotensive drug with anti-fibrotic properties; in mouse models, it has increased the distribution of some therapeutic nanoparticles by limiting extracellular matrix. We performed in-vitro experiments to verify losartan's suitability as a combination treatment with oncolytic adenoviruses. Toxicity tests showed that losartan had no anti-cancer properties of its own. Various cell lines in culture were then treated with a combination of losartan and adenovirus.

In several cell lines, the combination treatment of losartan and adenovirus was markedly more effective than the adenovirus alone. H1299 cells responded particularly well to the combination treatment, with positive results in each of the four analytical methods used. We showed that losartan increases the initial viral penetration and distribution. Virus titers suggested losartan increases viral replication; losartan also exhibits a positive effect on viral cytotoxicity and the efficacy of adenovirus therapy.

Losartan appears to be a viable option to increase adenovirus distribution in tumors. More testing is needed to understand the full range of Losartan’s effects and how to maximize its positive effect on viral efficacy. This research was supported by NCI grants R01 CA129975 and R25-CA134283.

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Title: Inhibition of S-adenosylhomocysteine hydrolase (SAHH) Induces Fas Ligand Gene Expression and Apoptotic Death in Leukemic T Lymphocytes [2014]

Authors: Harold Ghooray,[1] Smita Ghare, Madhuvanti Patil, Swati Joshi-Barve, Craig McClain , Shirish Barve .[1] Medicine[1] and Pharmacology & Toxicology.[2]

Keywords: S-adenosylhomocysteine, Fas Ligand, Leukemic T Lymphocytes, Transmethylation Pathway

Abstract:

The transmethylation (TM) pathway is up regulated in activated proliferating T cells and transformed T leukemic cells but not in resting T cells, making it an ideal target to eliminate leukemic T cells. The TM pathway involves the methyltransferase-mediated donation of methyl groups by S-adenosylmethionine (SAM) and conversion of SAM to S-adenosylhomocysteine (SAH). SAH is a potent feedback inhibitor of methyltransferases and has the potential to influence DNA and histone methyltransferases affecting chromatin remodeling events that dictate gene expression. Under physiologic conditions SAH is hydrolyzed to adenosine and homocysteine by S-adenosylhomocysteine hydrolase (SAHH) which catalyzes the only reversible reaction in the TM pathway.

The present work was carried out to examine the effect of inhibiting SAHH on the survival of T leukemic cells. SAHH was inhibited in T cell leukemic cell lines – Jurkats and Molt-4 by using two distinct pharmacological agents - 3-deaza-adenosine (DZA) and 3-Deazaneplanocin A (DZnep). The data obtained showed that SAHH inhibition markedly decreases the cellular methylation potential and induces apoptotic death in T leukemic cells. Analysis of the molecular mechanisms underlying the apoptotic death demonstrated that SAHH inhibition leads the induction of FasL gene expression. We are currently examining the chromatin changes in the promoter region of the FasL gene that are induced by SAHH inhibition in T leukemic cells. Overall, the data indicate that SAHH could be a potential therapeutic target in the treatment of T leukemic cells. This research was supported by the National Cancer Institute grant R25-CA134283.

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Title: Biomarker Significance of Exosomes in the Initiation and Progression of Breast Cancer [2014]

Authors: Kendall Huddleston,[1] Farrukh Aqil,[2] Ramesh Gupta,[1] Radha Munagala.[2]
Department of Pharmacology and Toxicology [1] and James Graham Brown Cancer Center.[2]

Keywords: Breast, Cancer, Exosomes

Abstract:

Currently, breast cancer diagnosis involves invasive methods such as mammography and biopsy. Thus, there's a need for non-invasive, patient-friendly methods. Recent developments indicate much promise for the use of circulatory serum exosomes as potential biomarkers for diagnosis. Exosomes are found in most bodily fluids, and are more abundantly released from tumor cells compared to healthy cells. We examined if serum exosomes could be used as potential biomarker during the initiation and progress of breast cancer using ACI rats, which develop tumors upon low-dose continuous exposure to 17β-estradiol (E_2). Serum from E_2-treated and control animals after 3 weeks, 3 months, and 7 months was used to isolate exosomes. Exosomes were analyzed for size, protein yield, and exosomal surface and proliferation markers. Exosomes were in the size range of 40-200 nm. The mean exosomal protein yield from E_2-treated samples was higher than the control for each time period; however, the differences were insignificant due to small sample size. Serum exosomes were positive for hallmark exosomal proteins including Alix, CD44, CD63, CD81, and proliferation marker, EGFR. Levels of Alix and CD44 were elevated in E_2-treated serum exosomes versus control. E_2-treated serum exosomes enriched using CD63-captured magnetic beads exhibited higher protein yield and EGFR expression than controls; however, the differences were insignificant. Higher levels of serum exosomes positive for exosomal surface proteins and proliferation markers with E_2-treatment indicate their potential as biomarker during initiation and progression of breast cancer. (Supported from NCI grant R25-CA134238, Duggan Endowment and Hemsley Funds).

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Title: The Role of Carbon Chain and Carbonyl Group in AHL-induced Caspase-9-dependent Apoptosis [2014]

Authors: Jaison John,[1] Nicole Stivers,[2] Aaron Neely,[2] Guoping Zhao,[2] John Eaton,[2] Chi Li.[2] Other[1] and Medicine.[2]

Keywords: Homoserine lactone , apoptosis, autophagy

Abstract:

N-Acyl-Homoserine lactones (AHLs) are essential quorum-sensing molecules that play vital roles, such as regulating virulence factors in pathogens. Our interest has been sparked by C12, an AHL produced by the opportunistic bacterium, *Pseudomonas aeruginosa*. We have recently discovered that C12 induces human tumor cell apoptosis, following a pathway that is caspase-9-dependent. Recent studies have also found that it preferentially kills transformed cells over normal cells. For this study, our goal was to identify other AHLs that induce caspase-9-dependent apoptosis. WT and Caspase-9 KO Mouse Embryonic Fibroblast (MEF) cells were treated with increasing concentrations of various derivatives of C12 for 24 hours. We found that reduction of the carbon chain length by treating with C6 and C8, eliminated the ability to induce cell death based upon the propidium iodide (PI) assay. However, C14, with increased carbon chain length, like C12 was able to induce the apoptosis cascade. Simply removing the carbonyl group from C14, forming C14 HSL, resulted in a completely different cell death pathway independent of caspase-9. We suspect that pathway to be autophagy, as many of the cells have an accumulation of LC3-B positive autophagosomes and are not Annexin V or PI positive. Our results indicated that both the carbon chain length and carbonyl group are important for AHL-induced caspase-9-dependent apoptosis.

Supported by grant R25-CA-134283 from the NIH/NCI.

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Title: Cannabigerol Modulates the Efficacy of Cannabinoids on CB2 Receptor [2014]

Authors: Alyssa Laun,[1] Pritesh Kumar,[1] Zhao-Hui Song.[1] Pharmacology and Toxicology.[1]

Keywords: cannabigerol, cannabinoid, CB2

Abstract:

Cannabigerol (CBG) is a non-psychoactive phytocannabinoid. It is currently unknown what the interaction of CBG is with the cannabinoid receptor 2 (CB2). This project measured the modulation of CBG on the effects of known cannabinoid agonists including endo- and synthetic cannabinoids. A homogeneous time resolved fluorescence method was used to quantify CB2 mediated decrease in cyclic adenosine monophosphate (cAMP) levels. CBG by itself had no effect on cAMP levels. However, CBG was found to increase the efficacy of AEA and WIN55,212-2, but no effect was observed on the other cannabinoids. This research was partially supported by NCI grant R25 CA134283 to the University of Louisville

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Title: Interaction of RIT and NORE1A in lung cancer [2014]

Authors: Michael A. Mannen,[1] M. Lee Schmidt, MS,[2] Geoffrey J. Clark, Ph.D.[3] Biology and Chemistry,[1] Biochemistry and Molecular Biology[2] and Pharmacology and Toxicology.[3]

Keywords: Lung Cancer, Cancer Molecular Biology

Abstract:

Lung cancer remains the leading cause of cancer related deaths worldwide. The most well characterized oncoprotein in lung cancer is the Ras Protein. Recently, other members of the Ras Superfamily have been indentified as being mutated in lung cancer cases and potentiallly may play a critical role in lung carcinogenesis. Prior to the summer, the Clark Lab performed a Yeast-2-hybrid screen which identified binding between RIT, Ras Superfamily member, and NORE1A, a well characterized tumor suppressor and negative effector of Ras. This summer project focused on determining if the interaction between NORE1A and RIT was conserved in mammalian cell lines and what were the relevant biological consequences of this interaction to lung cancer cell lines. The data presented demonstrates that NORE1A binds RIT proxmial to the plasma membrane, in a GTP to GDP independent manner in HEK 293 cells. Moreover, the specificity of this binding appears to be unique to RASSF5(NORE1A) and not other members of the Ras Association Domain Family Member Proteins. Luciferase assays using a PUMA reporter demonstrate that overly exogenously expressed RIT protein decrases NORE1A's transcription of pro-apopotic genes. A growth curve growth curve performed in h1299 cells selectively stabilized for +/- NORE1A/RIT show that RIT prevents NORE1A growth suppression in Non-Small-Cell lung carcinoma cell lines. These results collectively suggesst a potential mechanism for RIT mediated lung carcinogenesis.

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Title: Breast Cancer Diagnosed through the Mobile Mammography Van in Jefferson County, KY [2014]

Authors: Sarah Mudra,[1] Jianmin Pan, Ph. D ,[2] Shesh Nath Rai, Ph. D ,[2] Beth Carloss Riley, MD, FACP.[3] Medicine, Division of Oncology/Hematology ,[1] Bioinformatics and Biostatistics[2] and Medicine, Division of Oncology/Hematology.[3]

Keywords: Breast Cancer, Mobile Mammography, Race , Insurance, Age , Obesity , Family History

Abstract:

Introduction: Historically, since higher rates of breast cancer mortality occur among underserved populations, mobile mammography aims to target these groups. Demographic, clinical and biological characteristics were investigated in women diagnosed with invasive breast cancer (stages I-III) or DCIS via the Mobile Mammography Unit (MMU) in Jefferson County, KY from 2000-2010.

Methods: 21,857 individuals received 48,324 screening mammograms, 247 requiring biopsies. 169 individuals were excluded due to benign pathology, high-risk status or unavailable surgical pathology. 78 remained for analysis. Demographic data (age, race, insurance status) and clinical and biologic factors (histologic diagnosis, biologic subtype, stage, BMI, family history) were collected retrospectively for cancer diagnoses. Descriptive statistics (frequency, percentage) were produced for categorical variables using SAS procedure FREQ.

Results: Despite a majority of insured visitors (57%), most diagnosed were uninsured (63%). Consistent with screen-detected cancer statistics, DCIS represented 23% of diagnoses. Over 25% of cancers were present in 40-49 year-olds. Cancers were more likely to be ER/PR positive. Triple negatives represented 8%; 13% were Her2Neu positive. Consistent with mammography objectives, early stage cancer (stages 0-II) represented 60% while locally-advanced disease (stage III) represented 5%, although 35% of data remained unavailable.

Conclusions: Black women exhibited a higher density of breast cancer. Obesity and family history, known risk factors, were consistent with the database. However, results require cautious interpretation due to unavailable data and bias given the targeted populations. Benefits may outweigh risks for women aged 40-49 in this breast cancer dense region, despite recent controversy.

Supported in part by NCI grant R25-CA134283.

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Title: Identification of an Internal Reference microRNA from the Plasma of Multiple Cancer Types [2014]

Authors: Alexander Myers,[1] Henry Roberts,[2] Jonathan Rice,[2] Robert Eichenberger,[2] Susan Galandiuk.[2] Biology[1] and Surgery.[2]

Keywords: microRNA, biomarker, cancer, plasma

Abstract:

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MicroRNAs have been discovered to be small non-coding genes, around 22 nucleotides in length, that cause the degradation or inhibition of protein translation by messenger RNAs. In the process of developing a biomarker for cancer, an internal reference microRNA needs to be established to use as a comparison against dysregulated microRNAs.

We extracted the plasma from 6 sample cohort groups: 20 from colorectal cancer (CRC); and 10 from colorectal adenoma (CRAd), breast cancer (BC), lung cancer (LC), pancreatic cancer (PC), and the controls. 381 microRNAs were screened and a fixed cycle threshold of 0.03 was established. The means and standard deviations were calculated, for each microRNA under analysis. We determined that two microRNAs were less varied and better for selection as internal reference genes.

Five microRNAs were included within this analysis. The microRNAs that showed the least variability were U6 and miR-520d-5p. These two microRNAs had the smallest error bars and were the most stable. A combined average of the ΔCT values from U6 and miR-520d-5p were included to show the stability between them.

Overall, our study has analyzed 381 different microRNAs, in which only two have been selected as potential internal reference genes. We have concluded that with a combination of our two least varied microRNAs (U6 and miR-520d-5p); they are the best option to use as internal reference genes, when analyzing different dysregulated microRNAs that could act as biomarkers for colon cancer. These two microRNAs have shown to be the most stable across each spectrum of groups analyzed.

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Title: Plant-Made Cholera Toxin B Subunit, an Orally Active Anti-inflammatory Protein, in an Acute Colitis Mouse Model: Investigation of Effective Dose and Time of Administration. [2014]

Authors: Joshua Royal,[1] Bailey Nelson,[2] Keegan Baldauf,[3] Calvin Kouokam,[4] Matoba Nobuyuki .[3] Pharmacology and Toxicology,[1] School of Medicine,[2] Pharmacology and Toxicology [3] and Owensboro Cancer Research Program.[4]

Keywords: Colitis, Anti-inflammatory, Plant-Made, Cholera Toxin B Subunit

Abstract:

Background: Cholera toxin B subunit (CTB) is a component of a licensed oral cholera vaccine and has anti-inflammatory activity. We have previously shown that recombinant CTB produced in tobacco plants (CTBp) mitigates dextran sulfate sodium (DSS)-induced colitis in mice. Here, we analyzed the effective dose and timing of administration. **Methods:** C57BL/6J mice were exposed to 3% DSS on Day 0 – 7 and allowed to recover for 2 days before sacrifice. Mice were orally administered twice with 3 µg or 10 µg CTBp in three separate dosing regimens; 1) Day -14 and 0; 2) Day 0 and 3; and Day 3 and 6. Upon sacrifice, body weights and fecal samples were analyzed for a Disease Activity Index (DAI). Colon was isolated and analyzed by ELISA for cytokines (IL-1β, TNFα, and IFNγ), quantitative PCR for inflammatory gene expression, and histological scoring. **Results:** CTBp, both 3 µg and 10 µg, significantly decreased the DAI score when therapeutically dosed on Day 3 and 6. Additionally colon shrinkage was significantly prevented. By contrast, neither of the prophylactic regimens showed a significant effect, but a trend of protection was noted with 10 µg CTBp administered on Day 0 and 3. Cytokine levels, gene expression of inflammatory markers, and histological inflammation scores are currently being analyzed. **Conclusions:** Our data suggest that CTBp is most effective when dosed during an active inflammatory phase.

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Title: Inhibition of PFKFB3 and BRAFV600E may be an effective treatment for metastatic melanoma [2014]

Authors: Conor ONeill,[1] Julie ONeal ,[2] Jason Chesney.[3] Biochemistry ,[1] Brown Cancer Center[2] and Medicine.[3]

Keywords: Melanoma, Glycolysis, Metabolism, PFKFB3, PFK158, Vemurafenib

Abstract:

In human cancers, rates of glycolysis have been shown to increase up to 200 times in order to achieve high rates of proliferation and survival. One key regulator, 6-phosphofructo-2-kinase (PFKFB3) phosphorylates fructose-6-phosphate (F6P) to produce fructose 2,6-biphosphate (F26BP), a potent activator of 6-phosphofructo-1-kinase (PFK1), that regulates an irreversible step of glycolysis. Oncogenes and various tumor suppressor genes regulate PFKFB3 (i.e. PTEN, RAF/BRAF, Hif1α). A promising drug, PFK-158 that inhibits PFKFB3 is currently in Phase I clinical trials. About half of metastatic melanomas express a mutant form of B-RAF (BRAFV600E) and those patients are treated with Vemurafenib (VEM) or Dabrafenib; both are specific inhibitors of mutant B-RAF kinase activity. However, up to 50% of patients treated with VEM respond, but then relapse while the other 50% are intrinsically resistant to VEM. Since BRAFV600E promotes glucose metabolism, survival and growth, and regulates HiF1α, we hypothesized that BRAFV600E regulates glycolysis through PFKFB3. Knockdown of BRAFV600E with specific siRNA's mimicked the glycolytic effect of VEM including down-regulating PFKFB3. To our surprise, overexpressing BRAFV600E had little effect on PFKFB3 in cells expressing WT BRAF. Lastly, combination treatment with VEM and PFK-158 in VEM resistant cells that express BRAFV600E, resulted in synergistic cell death. Our data suggest VEM + PFK-158 may be a promising treatment option for metastatic melanomas resistant to Vemurafenib.

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Title: Circadian Disruption: distress and sleep quality in breast cancer patients [2014]

Authors: Thomas Packer,[1] Lauren Zimmaro,[1] Elizabeth Cash,[2] Sandra Sephton.[2]
Psychological and Brain Sciences[1] and J.G. Brown Cancer Center.[2]

Keywords: Breast Cancer , Circadian Disruption, Distress, Actigraphy

Abstract:

Breast cancer is the most common form of cancer and second leading cause of cancer deaths among women of all races. The distress accompanying a cancer diagnosis and its affect on circadian rhythmicity has not been fully explored. We tested for the predictability of circadian activity measures based on the psychosocial factor of distress. Our assumptions were based on a model of tumor progression (Eismann, et al., 2010). Forty-eight breast cancer patients completed an Impact of Event Scale self-report to measure their subjective response to their cancer diagnosis. Actigraphy is the recording of body movement that provides a noninvasive measure of rest-activity rhythms and sleep patterns. Participants wore a wristwatch-like device (Motionlogger) continuously for four days. Voltage is created by movement and is recorded in 60-second segments, creating a curve scored as “wake” or “sleep”. The circadian rhythm activity was estimated using the autocorrelation coefficient calculated based on 24-hour time lags. We hypothesized that total distress would be associated with circadian rhythmicity, sleep efficiency, amount of time spent awake after sleep onset, amount of time spent sleeping, and the number of nightly awakenings. After multiple regression analysis controlling for age and stage of diagnosis, there were no significant ($P<0.05$) findings to the support IES subscales Intrusion and Avoidance as predictors for circadian disruption. Total distress and sub scores reported from the IES did not show significant proportion of variance in circadian activity measures.

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Title: Plasma-Based microRNA Panel Specific for Colorectal Neoplasia [2014]

Authors: Henry Roberts,[1] Jonathan Rice,[2] Robert Eichenberger,[2] Jianmin Pan,[3] Shesh Rai,[3] Susan Galandiuk.[2] Medicine,[1] Surgery[2] and Bioinformatics and Biostatistics.[3]

Keywords: microRNA, Plasma, Colorectal Cancer , Adenoma, Biomarker

Abstract:

Introduction:

We have previously demonstrated plasma microRNAs to be dysregulated in patients with advanced colorectal adenomas (> 0.6 cm diameter) [CRAd] and colorectal cancer (CRC) as compared to controls. Numerous miRNAs have been reported in the literature to be significantly dysregulated in the plasma of many different cancer types (e.g. miR-21).Thus in developing a useful blood test for colorectal neoplasia, it is necessary to identify a test *specific* for colorectal neoplasia, as opposed to other common cancers.

Methods:

We screened for 381 miRNAs using microfluidic array technology (Applied Biosystems), in a discovery cohort of 20 CRC patients, 10 patients each with CRAd, breast cancer (BC), lung cancer (LC), pancreatic cancer (PC) and controls. Each miRNA was normalized to the mean of U6 and miR-520d-5p (ΔCT). Only microRNAs with >90% expression were included in statistical analysis. Statistical analysis identified 10 microRNAs specific for colorectal neoplasia.

Results:

Panel of 10 miRNAs permitted accurate differentiation of:

1) Controls from patients with any type of neoplasia (CRC, CRAd, BC, LC, PC), Area under the curve (AUC) = 0.863

2) Patients with colorectal neoplasia (CRC, CRAd) from patients with other common cancers (BC,PC,LC), AUC = 0.984

3) Patients with CRC from CRAd with an AUC of 0.909

Conclusions:

The discovery phase for our plasma miRNA panel specific to colorectal neoplasia shows tremendous potential. These data will be verified and validated using individual miRNA assays in a larger test cohort (n=120) and double blinded validation cohort (n=150), respectively

Acknowledgements:

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Title: Impact of Quercetin on miR-25 and Cellular Behavior in Prostate Cancer Cell lines [2014]

Authors: Angelica Ronke-Hervey,[1] Dominique Jones B.A.,[1] LaCreis Kidd Ph.D.[2] Pharmacology and Toxicology[1] and Pharmacology and Toxicology/ James Graham Brown Cancer Center.[2]

Keywords: microRNA, Prostate cancer, Quercetin

Abstract:

Background: Preliminary data from our lab demonstrated two oncomiRs (e.g., miR-106b and miR-186) were up-regulated in non-metastatic (E006AA) and/or metastatic prostate cancer (PCA) cell lines (e.g., PC3). The up-regulation of oncomiRs may be counteracted by various chemopreventive agents, including quercetin. *In vivo* studies demonstrated quercetin modulates the expression of miRNAs. However, it is not clear whether quercetin modulates the expression of miR-186 and the miR-106b-25 cluster (miR-106b, -25, -93).

Hypothesis: We hypothesized that quercetin will decrease cell proliferation and miR (-186 and -106b-25 cluster) expression in metastatic (i.e., PC-3) and non-metastatic (i.e., E006AA) PCA cell lines.

Methods: After cells were treated with quercetin (12.5, 25.0, 50.0, 75.0μM), the extent of cell proliferation at 24 hrs was assessed using the ATPLite Luminescence Assay. E006AA cells were plated into 6-well plates with five 2mm silicon plugs. After removing the plugs, the area was photographed every 4 hrs for 16 hrs to measure cellular migration using Image J software. miRNA levels were determined by qRT-PCR.

Results: Cell viability was reduced by 72% in E006AA cells treated with quercetin (75μM) compared to the 50μM treatment ($P < 0.0001$). In addition, quercetin treatment (75μM) in E006AA cells revealed a 47.4% reduction in cell migration. Consistent with our hypothesis, quercetin treatment (75μM) down-regulated miR-25 expression in PC3 cells.

Clinical Relevance: The findings of our study may serve as a foundation for future studies to identify and validate new treatment strategies for individuals susceptible to pre- and metastatic PCA.

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Title: Analysis of Mutant Epidermal Growth Factor Receptor Trafficking and Signaling in Lung Cancer Cells [2014]

Authors: Tejas Sangoi,[1] Adriana Bankston, Brian Ceresa. Cancer Education Program[1] and Pharmacology & Toxicology.[2]

Keywords: EGFR, Trafficking, Signaling, Cancer, Lung

Abstract:

Purpose: To better understand how activating mutants of epidermal growth factor receptor (EGFR) regulate trafficking and signaling of the receptor in lung carcinoma cells.

Methods: H1650 cells express mutant EGFRs; A549 cells express wild type EGFRs. Immunoblotting was used to test for time-dependent EGFR phosphorylation and degradation upon EGF stimulation. Receptor trafficking and endosomal accumulation at steady state were observed by immunofluorescence staining of the total EGFR. The pattern of EGFR constitutive recycling of the receptor was examined by incubating with Ab1 antibody. Kinetics of ^{125}I-EGF endocytosis and ligand-mediated degradation were measured by radioligand binding assay.

Results: Immunoblotting for phosphorylated EGFR shows an increase in steady state EGFR phosphorylation, as well as elevated phosphorylation of effector molecules MAPK and MEK in H1650 cells. Steady state distribution of unliganded, mutant EGFR through immunostaining indicates localization in the early endosome. After 24 hours of Ab1 treatment, mutant EGFRs show increased endosomal accumulation. Furthermore, mutant EGFRS exhibit a slower rate of recycling and degradation.

Conclusions: Activating mutants of EGFRs found in lung cancer cells have higher levels of phosphorylation in EGFR Y1068, MAPK, and MEK. Localization of mutant EGFR is seen in the early endosome at steady state, as well as a defect in constitutive membrane trafficking after 24 hours. Less radioligand recycling and degradation in the muntant lung cancer cell lines indicate a defect in endocytic trafficking.

Supported by National Cancer Institute grant R25-CA134283 and National Institutes of Health grant R01-GM092874.

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Title: Effects of Nanoparticle Morphology and Surface Modification on Tumor Penetration and Distribution [2014]

Authors: Lee Sims,[1] Hermann Frieboes,[1] Jill Steinbach.[1] Bioengineering.[1]

Keywords: PLGA nanoparticle , tumor spheroid

Abstract:

One of the challenges to efficacious drug and gene delivery to solid tumors is inadequate penetration and distribution throughout the tumor vasculature. To overcome these challenges, nanotechnologies such as nanoparticles (NPs), can be utilized to protect agents during delivery, prolong delivery, and safely localize drugs and biologics to the tumor microenvironment. In addition to these attributes, NPs can be modified to enhance penetration and distribution throughout the tumor vasculature. Different factors including: NP surface charge, surface composition, size, and shape are integral to enabling drug delivery vehicles to withstand systemic interactions and to transport to the tumor site. The long-term goal of this study is to develop adaptable poly(lactic-co-glycolic acid) PLGA NPs with a variety of surface modifications and sizes to evaluate how each modification contributes to 3-D distribution in a tumor spheroid model. In combination with mathematical modeling to predict formulations that will enhance distribution, experimental validation will enable us to rationally design NP formulations that successfully penetrate the tumor microenvironment. We hypothesize that ultra-small (< 70nm) and/or surface-modified NPs will improve targeting of, and penetration to the tumor spheroid, and will significantly enhance NP uptake to individual cells. The experiments conducted here provide us with a preliminary assessment of design factors governing NP-tumor interactions in a 3-D environment. We expect these and future experiments will provide insight to select efficacious modifications for increased tumor targeting and distribution.

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Title: The role of the BH-4 domain in dictating the oncogenic potency of BCLxl [2014]

Authors: Amy Song,[1] Levi Beverly.[1] Medicine.[1]

Keywords: BCLxl, BCL-2, MOMP, BH-4 domain

Abstract:

BCLxl, a member of the B-cell lymphoma-2 (BCL-2) family, is a protein that plays a key role in cell survival by preventing mitochondrial outer membrane permeabilization (MOMP). The ability of BCLxl to block apoptotic signals in the cell has been positively linked to tumorigenesis. Earlier in vivo experiments in the lab using mouse models showed the significance of individual domains within BCLxl's protein structure for dictating oncogenic potency. The Bcl-2 homology (BH)-4 domain of BCLxl has been shown to be essential in the anti-apoptotic functionality of BCLxl and certain residues within the BH4 domain of Bcl-2 are significant to the pro-survival potency of the protein. Site-directed mutagenesis was used to induce point mutations of conserved residues within the BH4 domain of BCLxl. Successful mutations would be used in ongoing in vitro experiments to further analyze the biochemical functions of BCLxl.

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Title: Recombinant Expression of Codon-Optimized ANAPC2 and ANAPC11 [2014]

Authors: James Stewart,[1] Mark Doll,[1] J. Christopher States.[1] Pharmacology and Toxicology.[1]

Keywords: Mitotic Arrest, Anaphase Promoting Complex, Codon Optimization, ANAPC2, ANAPC11, Recombinant Protein

Abstract:

Current mitosis disrupting chemotherapeutics on the market today are spindle poisons that attack mitotic spindle formation and activate the spindle assembly checkpoint (SAC). Activation of the SAC inhibits the Anaphase Promoting Complex/Cyclosome (APC/C), an E3 ubiquitin ligase. Prolonged mitotic arrest leads to cell death. Direct inhibition of the APC/C will lead to mitotic arrest and could eliminate the need for a functional SAC. APC/C inhibition by disrupting interaction between its subunits ANAPC2 and ANAPC11 will lead to loss of function and cause apoptosis. Using homology structures for in silico screening identified several compounds that could disrupt ANAPC2/ANAPC11 binding. Thermoflour assays can be used in order to show the on target binding of these compounds and give an order of binding affinities. Previous attempts at expression and purification of recombinant ANAPC2 and ANAPC11 using human cDNA sequences failed. Therefore, codon usage in the ANAPC2 and ANAPC11 cassettes was optimized for E. coli. Codon optimized recombinant ANAPC2 and ANAPC11 cassettes were cloned into the IMPACT expression system as N-Terminal constructs in pTYB21. IPTG concentration and growth temperature and time were tested in order to find optimal expression conditions. Production of purified recombinant ANAPC2 will allow binding assays to be run to test compound binding affinities. Compound binding to ANAPC2 with high affinity may displace ANAPC11 from ANAPC2/ANAPC11 complexes. Evidence of displacement will demonstrate the APC/C as a new target for future chemotherapeutic drugs. Partially supported by NCI grant R25 CA134283 to the University of Louisville.

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Title: Evaluation of coated gold nanoparticles targeted with Syndecan-1 for detection of pancreatic adenocarcinoma [2014]

Authors: Chris Ullum,[1] Shanice Hudson,[1] Anil Khanal,[1] Lacey McNally.[1] Department of Medicine.[1]

Keywords: Gold nanorod, MSOT, Syndecan-1, Pancreatic, Cancer

Abstract:

Purpose: Mortality of pancreatic cancer remains unchanged for the past four decades due in part to the inability to detect early stage tumors. We sought to create a contrast agent which would identify pancreatic tumor cells using a newly emerging imaging system, Multi-spectral Optoacoustic Tomography (MSOT). We developed two fully functional and stable gold nanorods to serve as this contrast agent. Methods: Gold nanorods (GNRs) were synthesized via the seed-mediated method. To overcome the common detriment of gold nanoparticle aggregation, the GNRs were coated with mesoporous-silica (MS) or poly-acrylic acid (PAA) and conjugated to Syndecan-1. Synthesized nanoparticles were characterized by Transmission Electron Microscopy (TEM), UV-Visible Spectroscopy (UV-vis), Zeta-potential, and Cytoviva Hyperspectral Imaging. Assessment of coated and targeted GNRs as potential contrast agents was determined using tissue phantoms and mice bearing orthotopic pancreatic tumors using MSOT imaging. Results: The GNRs have an aspect ratio of 30:7 with MS-GNR and PAA-GNR containing a 10 nm mesoporous silica shell and 3 nm PAA shell. Upon coating of MS-GNR and PAA, the UV-vis spectra showed a red-shift of 32 nm and 15 nm. Neither the MS-GNR nor PAA-GNR aggregated at pH 7.4. The Syndecan-1 MS-GNR particles were detected on the cellular membrane of pancreatic cancer cells. Syndecan-1 MS-GNR particles facilitated detection of orthotopic pancreatic via MSOT in mice. Conclusion: Syndecan-1 targeted MS-GNR could facilitate detection of pancreatic tumors using Multispectral Optoacoustic imaging in both preclinical and clinical settings. Further studies will be conducted to conclude the biodistribution and pharmacokinetics of Syndecan-1 MS-GNR in vivo.

National Cancer Institute grant R25-CA134283

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Title: Modulating Epidermal Growth Factor Receptors via Small Targeting Peptides [2014]

Authors: Adrienne Voelker,[1] Luis Neves,[1] Brian Ceresa.[1] Department of Pharmacology and Toxicology.[1]

Keywords: EGFR, liposomes, endocytic pathway, peptide

Abstract:

The purpose of this project is to better understand the epidermal growth factor receptor (EGFR) in the endocytic pathway and the effect of a small Sprouty2-derived peptide on the rate of degradation of EGFR, which impacts cell proliferation and wound healing.

Liposomes were synthesized and peptide (FITC-Ahx-IRNTNE{pTYR}TEGPTV) was encapsulated in the liposomes via freeze and thaw cycles. Liposomes were incubated with the S2VP10 human pancreatic cancer cell line. S2VP10 cells were also treated with digitonin to permeabilize the membrane and incubated with peptide at different concentrations. Efficiency of peptide uptake into the cell was determined by fluorescent microscopy. EGFR degradation levels upon EGF ligand stimulation at time points 0, 15, 60, and 120 minutes were measured through immunoblotting.

Dynamic Light Scattering (DLS) and spectral evaluation of the liposomes confirm that synthesis of the liposomes and encapsulation of the peptide was successful. Fluorescent microscopy shows that liposomes offer an efficient delivery of the peptide into the cell. EGFR activity levels, quantified through immunoblotting, do not suggest an effect induced by the liposomes.

The methodology used for the synthesis and peptide encapsulation allows for successful encapsulation of the peptide. Liposomes are an effective method of delivering the peptide into the cell in a controlled, targeted manner. Western Blot results do not reveal a significant effect of the peptide on EGFR activity levels in the cells. Future experimentation is required to determine the efficacy of the peptide.

This project was funded by National Cancer Institute grant R25-CA134283.

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Title: Is There a Relationship between Patient Worry and Preferences for Follow-up Care after Curative Treatment for Lung Cancer? [2014]

Authors: Ariel Washington,[1] Karen Kayser,[2] Smita Ranjan,[3] Lisa Smith,[2] Goetz Kloecker.[4] Physiology and Biophysics,[1] Social Work,[2] James Graham Brown[3] and Medicine.[4]

Keywords: Patient preferences, worry, lung cancer, follow-up care

Abstract:

Kentucky has the highest rate of lung cancer in the nation. Not only is the incidence high, the survivorship rate is low at a rate of 16.18% (American Lung Association). Despite the medical advances in treatment, there is still no standard, unified guideline for follow-up surveillance of patients after curative treatment ends.

We hypothesize that patients who experience a higher level of worry about cancer will desire more frequent follow-up appointments by their providers and more discussion about their psychological distress and information about their cancer during their appointments.

Lung cancer patients who were deemed cancer-free after curative treatment were surveyed about their medical and supportive care preferences while also answering questions about their fear of reccurrence. Their responses indicated a desire for more information about cancer and future planning along with information addressing their psychosocial needs. The analysis revealed significant correlations between these preferences and respondents' level of worry. In particular, patients who reported high levels of cancer worries were more likely to prefer follow-up visits that included discussions about their coping with cancer and receiving information about supports available for their family. Patients with high worry were more likely to prefer their follow-up visits to occur at a shorter interval.

Although we present preliminary findings from a small sample, the results indicate some significant correlations between patient worry and their preferences for medical and psychosocial follow-up care. Our future research plans will also examine physician preferences for patient follow-up and the development of guidelines for surveillance decision-making.

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Title: Examination of the Effects of Small Molecule Inhibition of PFKFB4 on the Cell Cycle [2014]

Authors: Lindsey Wattley,[1] Jennifer Clark,[1] Erin Ballard,[1] Sucheta Telang.[1] Med (Hem-Onc).[1]

Keywords: cell cycle, PFK, glycolysis

Abstract:

A high rate of glycolysis despite the presence of oxygen *(i.e.* the Warburg effect) is a metabolic hallmark of cancer cells. Fructose-2,6-bisphosphate (F2,6BP) is a shunt product of glycolysis which activates a key glycolytic enzyme, 6-phosphofructo-1-kinase (PFK-1). F2,6BP is produced by the family of 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatases (PFKFB1-4) that interconvert fructose-6-phosphate and F2,6BP. The PFKFB4 isoform has been found to be increased in multiple tumor types and silencing PFKFB4 has been shown to decrease intracellular F2,6BP and inhibit cancer cell growth in culture and in mice. Through computational modeling, a small molecule inhibitor of PFKFB4 (5MPN) has been identified which decreases the growth of several lung cancer cell lines. We have observed that 5MPN decreased cell proliferation by arresting cancer cells in the G0/G1 stage of the cell cycle and the goal of our studies was to compare these effects to those of siRNA-mediated PFKFB4 knockdown. We first knocked down PFKFB4 using siRNA in two lung cancer cell lines (H460, H1299) and found that treatment with siRNA targeted to PFKFB4 for 24 hours caused an arrest of the cell cycle in G0/G1 phase similar to that caused by treatment with 5MPN. We then transduced H460 and H1299 cells with a plasmid expressing PFKFB4 and found that the inhibition of growth caused by 5MPN was reversed by the ectopic expression of PFKFB4. Future studies will examine the effects of shorter periods of exposure to 5MPN on cell cycle progression.

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