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Title: Lunasin Soy Peptide Inhibition of Melanoma in-vitro [2013]

Authors: Matthew Bienick,[1] Christopher Shidal, B.S.,[2] Keith Davis, Ph.D.[1] Pharmacology and Toxicology[1] and Pharmacology and Toxicology .[2]

Keywords: Lunasin, Melanoma, Soy Peptide

Abstract:

We are currently investigating the effects of lunasin's ability to inhibit the proliferation of melanoma cells *in vitro*. Lunasin, a soy peptide of 44 amino acids, decreases proliferation of several cancer types. Studies have revealed its mechanism of action is through interactions with core histone tails (i.e. H3 and H4), where it binds to lysine residues to competitively inhibit binding of histone acetyltransferases involved in activation of transcription.

PLX4032 (Vemurafenib) is a B-RAF inhibitor of the MAPK pathway. PLX4032 is able to selectively arrest the proliferation of cells that contain this mutant form of B-RAF. Unfortunately, clinical research has shown that after initial tumor regression in patients responding to treatment, tumors metastasize and progress with little sensitivity to ongoing treatments.

It is hypothesized that combination treatment of lunasin with PLX4032 will produce a synergistic antiproliferative effect on selected cell lines. Melanoma cell lines were chosen to have the V600E mutation. We expect lunasin and PLX4032 to interact cooperatively to abolish the ability of melanoma cell lines to form tumors and to sensitize resistant strains to vemurafenib treatment.

Initial results suggest that lunasin and PLX4032 interact synergistically in soft agar colony forming assays and additively in spheroid assays. Calculated Drewinko Index (DI) values reveals a broad spectrum of interactions between lunasin and vemurafenib, which is reliant upon dose and cell line. Future experiments are needed to determine the exact mechanisms of this interaction and extrapolate clinically relevant data.

Supported by grant R25-CA-134283 from the National Cancer Institute

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Title: Acetylator genotype-dependent N-acetylation of isoniazid in human hepatocytes in situ [2013]

Authors: Srineil Bodduluri, B.S., B.A., Mark A. Doll, M.S., Raul Alejandro Salazar Gonzalez, M.S., David W. Hein, Ph.D. Pharmacology & Toxicology.[1]

Keywords: Pharmacogenomics, Tuberculosis, Isoniazid, NAT2

Abstract:

Hepatotoxicity is a common symptom among patients undergoing treatment for tuberculosis that may progress to liver cancer. Clinical studies have recommended that a genotype dependent “pharmacogenetic” approach be used in determining a dose required for successful treatment and minimization of toxicity. N-acetyltransferase 2 (NAT2) is the primary enzyme involved in the metabolism of the anti-tuberculosis drug isoniazid and is subject to genetic polymorphism in human populations resulting in rapid, intermediate, and slow acetylator phenotypes. NAT2-genotype-dependent metabolism of isoniazid to date has not been observed in liver hepatocytes in situ. *NAT2* genotype in the cryopreserved hepatocytes was determined by Taqman allelic discrimination assay of genomic DNA. Cryoplateable human hepatocytes from three rapid NAT2 acetylator genotype (*NAT2*4/*4*, **12A/*12A* and **4/*4*), intermediate acetylator genotype (*NAT2*4/*6A, *4/*5B* and **4/*7B*), and slow acetylator genotype (*NAT2*5B/*5B, *5B/*6A* and **6A/*6A*) were thawed, plated and equilibrated 24 hr prior to isoniazid exposure. The production of acetyl-isoniazid in the media was measured by high performance liquid chromatography up to 48 hr. Human hepatocytes cultured in situ demonstrated a dose-dependent (10-200 µM) and time-dependent (0 - 48 hrs) increase in acetyl-isoniazid. When comparing the amount of acetyl-isoniazid produced/24hr/millions cells among the various samples, a robust and significant ($p<0.001$) NAT2 acetylator genotype dependent difference was observed in production of N-acetyl-isoniazid, with rapid NAT2 acetylator heptatycyes producing the highest amounts of acetyl-isoniazid followed by intermediate acetylators and slow acetylators following exposures to both 12.5 and 100 µM isoniazid. Partially supported by National Cancer Institute grant R25-CA-134283.

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Title: Gene Expression Alterations in Breast Carcinomas from Patients with Racial Differences [2013]

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

Keywords: Racial differences, Gene Expression, Breast cancer

Abstract:

African-American women often exhibit aggressive breast cancers and higher mortality rates than Caucasian women. Our goal is to determine dissimilarities in gene expression of breast carcinomas of white and black patients and relationships to cancer behavior. Gene expression levels were compared in patient biopsies employing microarray analyses of LCM-procured carcinoma cells. Frozen tissue sections of primary carcinomas were utilized for qPCR analyses. Total RNA was extracted with RNeasy (Qiagen), evaluated with the Bioanalyzer (Agilent) and reverse transcribed using iScript (Biorad). qPCR was performed using Power Sybr Green (ABI) and relative expression was calculated using Universal Human Reference RNA (Stratagene) and ACTB for normalization. Microarray revealed expressions of CARD11, TRAPPC2L, CRYBB2P1 and PDHA1 were significantly different in carcinomas of African-American patients compared to Caucasians. PDHA1 expression correlated with overall survival (p=0.05) when 245 patients were stratified by median expression level without regard to race. PDHA1 expression assessed by microarray also correlated with overall survival of white patients (p=0.04) stratified by race and gene expression. When patients were stratified by median expression levels, PDHA1, CRYBB2 and TRAPPC2L were not significant for associations with disease-free or overall survival. However, when stratified by race and median gene expression, increased expression of TRAPPC2L was correlated with longer disease-free survival (p=0.002, HR=8.84) in black patients. These preliminary results support a need for identifying biomarkers to personalize clinical management of breast cancer patients with different racial backgrounds. Supported by the R25 NCI Summer Research Program, PHS Grant #1 R25 CA134283-1.

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Title: A microgenomic approach to identify clinically relevant gene signatures that discriminate between invasive lobular and ductal breast carcinomas [2013]

Authors: Sean Butterbaugh, Sarah Andres, Ph.D., Mary Ann Sanders, M.D., Ph.D.,[2] James L. Wittliff, Ph.D., M.D. hc. Biochemistry & Molecular Biology[1] and Pathology.[2]

Keywords: inasive lobular carcinoma, invasive ductal carcinoma, genomics, breast cancer

Abstract:

In an effort to distinguish between the two most common invasive breast carcinomas, lobular (ILC) and ductal (IDC), we searched for a genomic marker that discriminates these pathologies when conventional tests are conflicting. A specific genomic marker is needed to easily distinguish IDC from ILC, due to the varied responses of luminal A-like-IDC and ILC to the aromatase inhibitor letrozole in post-menopausal women [Metzger et al. Cancer Res 2012]. To identify candidate genes, microarray analysis of expression levels were evaluated in laser capture microdissected carcinoma cells of biopsies that were positive for estrogen (ER) and progesterone receptors (PR). In low grade IDC and ILC, 299 probes were differentially expressed ($p<0.01$), and 99 of these probes were not differentially expressed ($p>0.01$) between high grade IDC and ILC. These 99 genes serve as candidates for a genomic marker differentiating the pathological subtypes. Microarray results showed varying expression levels of BRWD1, CAPSL, CHRNA, CMTM7, CRMP1, GSKIP, HBEGF, PAPPA, and LRBA among the different cancer pathologies. By using quantitative polymerase chain reaction (qPCR), we determined expression levels relative to ACTB for the gene candidates in order to validate those from the microarray array results. qPCR analyses are used to validate and refine the gene subset distinguishing ILC from low grade IDC. Our novel approach is revealing microgenomic features that discriminate these carcinomas which exhibit different clinical behaviors. Supported in part by a grant from NIH/NCI R25-CA134283 and Phi Beta Psi Charity Trust.

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Title: Role of Exosomes in Tumor Growth and Metastasis [2013]

Authors: Cameron Campbell,[1] Radha Munagala, Ph.D.,[2] Ramesh Gupta, Ph.D.[1] Other[1] and Medicine.[2]

Keywords: Exosome, Metastasis

Abstract:

Exosomes are endosome-derived 30-100 nm biological nanoparticles released from all types of cells but in abundance from tumor cells. Tumor-derived exosomes have been reported to be taken up by cells other than those of their origin and facilitate tumor growth, metastasis, and drug resistance. We hypothesized that treatment of non-metastatic lung cancer cells with exosomes from metastatic lung cancers would increase growth rate, migratory behavior, and invasiveness of the non-aggressive cancer cells. To asses this hypothesis, non-metastatic lung cancer cells (H522) were treated with exosomes from metastatic cancer cells (H1299 and A549) and were monitored for uptake of exosomes, wound healing, migration, and invasion capabilities. Western Blot analysis was performed on exosome-treated H522 cells to determine the expression levels of proteins essential for epithelial-mesenchymal-transition (EMT) leading to metastasis. Our results suggested that exosome treated H522 cells exhibited faster growth rate, increased migratory behavior, and invasiveness. EMT-associated protein expressions were also found to be modulated in a consistent manner for an EMT shift in the exosome treated H522 cells. These findings indicate that exosomes could play an important role in the development and metastasis of lung cancer. This may provide researchers a potential target to prevent metastatic cancers either by blocking exosome release from cancer cells or inhibiting uptake of exosomes by normal cells.

Supported by grant R25-CA-134283 from the National Cancer Institute.

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Title: Role of Tumor Cell – Stromal Interactions on TGFβ-1 Mediated Gene Expression [2013]

Authors: Matthew Cook,[1] Jeffrey Ritzenthaler,[2] Jesse Roman.[2] Pharmacology and Toxicology, University of Louisville[1] and Medicine, University of Louisville.[2]

Keywords: TGFβ-1, extracellular eatrix, SMAD3, a-SMA, lung cancer

Abstract:

The extracellular matrix (ECM) plays an important role in the growth, proliferation, and metastasis of tumor cells. These effects have been linked to the activity of the cytokine transforming growth factor beta (TGFβ-1). TGFβ-1 has also been shown to be involved with the development and progression of lung cancer, the leading cause of cancer-related deaths in the United States. What is not completely understood is how TGFβ-1 and the ECM interact to promote cancer development. We hypothesize that tumor cell–stromal interactions modulate TGFβ-1 induced gene expression, and tested these interactions in murine primary lung fibroblasts and in Lewis Lung Carcinoma (LLC). First, we showed that TGFβ-1 stimulated the proliferation of cells and that this was affected by the ECM. Second, we showed that LLC cells expressed SMAD3 and a-SMA mRNA in response to TGFβ-1 and that this was enhanced when cells were grown on the ECM molecule fibronectin (FN). Third, Western blot analysis showed that SMAD3 protein levels were further elevated in TGFβ-1 treated LLC and fibroblast cells grown on collagen type I (Coll I) and FN. Importantly, we found that SMAD3 nuclear binding to the a-SMA gene promoter increased in TGFβ-1 treated cells, and this was modulated by ECM. We conclude that ECMs indeed influence TGFβ-1/SMAD3 – dependent gene expression.

This work was supported by: NIH/NCI grant R25-CA134283.

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Title: Detection of Pancreatic Adenocarcinoma in vivo with S100A9 Liposomes [2013]

Authors: Tess Dupre, BS,[1] Christopher England, MS,[1] Justin Huang, BS,[2] Lacey R. McNally, PhD.[3] Pharmacology and Toxicology,[1] Medicine[2] and Oncology.[3]

Keywords: pancreatic cancer, drug delivery

Abstract:

Purpose: Delivering effective drugs at effective concentrations to all cells in solid tumors, especially pancreatic adenocarcinoma, remains challenging due to low drug accumulation within tumors and high levels of systemic toxicity. To overcome these problems, S100A9 liposomes can be utilized to enhance drug delivery. We hypothesize that S100A9 liposomes will exhibit enhanced accumulation in pancreatic orthotopic tumors due to S100A9 specificity for the EMMPRIN receptor.

Methods: Pancreatic cell lines, S2VP10, MiaPaCa2, S2CP9, and Panc-1, were evaluated for EMMPRIN expression along with ES2 (positive control) and MCF7 (negative control) using western blot. Liposomes were bioconjugated with S100A9 and CF750 NIR dye. Specificity and activity of the S100A9 liposomes were studied using flow-cytometry. SCID mice were orthotopically implanted with S2VP10 cells. After tumors reached 3mm, 200 uL of 5 OD S100A9 liposomes was IV injected into mice. Mice were imaged using 2D AMI imaging and 3D MSOT.

Results: EMMPRIN expression was seen at 1.2X and 0.9X relative abundance in S2VP10 and MiaPaCa-2 cells respectively; however, MCF7 expressed 0.1X relative abundance EMMPRIN. Flow-cytometry showed cellular uptake of S100A9 liposomes in S2VP10 and MiaPaCa2 cells at ~92% and ~84% respectively. 3D MSOT imaging and 2D AMI imaging showed accumulation of the S100A9 liposomes at the tumor.

Conclusion: The S100A9 liposome selectively targeted pancreatic orthotopic tumors *in vivo*, thus establishing that the stealth liposomes can be used as a drug delivery system for an alternative pancreatic cancer treatment.

Acknowledgements: Supported by R25-CA-134283 and CA139050.

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Title: Differential Expression of Chemokine Receptors in Lung Cancer Metastasis [2013]

Authors: Taylor Hermann,[1] Rajesh Sharma, PhD,[2] Zinal Chheda, MS,[3] Haribabu Bodduluri, PhD.[4] Medicine,[1] Microbiology,[2] Biotechnology[3] and Immunology.[4]

Keywords: Chemokine, Receptor, Lung Cancer, Metastasis, CCR, CXCR

Abstract:

Metastasis accounts for most cancer related deaths. Commonly used metastasis models bypass the egression of malignant cells in metastasis of spontaneous cancers. We developed a unique metastasis model by *in vivo* passaging the Lewis lung carcinoma (3LL) cells and maintaining them *in vivo* with 7-11 days of intermittent culture between passages. When injected subcutaneously, parental 3LL cells have minimal metastasis, while *in vivo* passaged 3LL cells (p-3LL) show robust metastasis in mice. Chemokine receptors have been found to play an important role in the migration of the cancer cells. We studied the expression profile of every chemokine receptor to discover which is most important in 3LL metastasis. Real-time PCR was used to examine the transcript levels of every chemokine receptor in 3LL and p-3LL cells and tumors. CCR6 and CXCR7 were found to be up-regulated in the passage tumors compared to the parental tumors, possibly indicating these receptors play a pro-metastatic role in cancer cells. CCR1 and CCR3 were down-regulated in the tumors, possibly indicating these receptors either play an anti-metastatic role or no role whatsoever. The chemokine receptors CCR3, CCR4, CCR9, and CXCR4 were up-regulated in the p-3LL cells as compared to the 3LL cells, indicating that these may be important in promoting metastasis. The chemokine receptors CCR1, CCR6, CXCR1, CXCR7, CX3CR1, and BLT1 all showed a significant reduction in the p-3LL cells, possible indicating these receptors metastasis of the p-3LL cells.

Acknowledgements: Supported by grant R25-CA134283 from the National Cancer Institute

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Title: Validation of Primary Non Small Cell Lung Cancer Cell Line: Stable Isotope Resolved Metabolomics Reveals Functional Biochemistry [2013]

Authors: Connor J Kinslow,[1] Teresa W.-M. Fan, Ph.D.,[2] Jin Lian Tan, M.S.,[1] Pawel K Lorkiewicz, Ph.D.,[2] Ramya Balasubramaniam, M.S.,[2] Andrew N Lane, Ph.D.[1] James Graham Brown Cancer Center[1] and Department of Chemistry.[2]

Keywords: SIRM, Metabolomics, Non-Small Cell Lung Cancer, C-MYC

Abstract:

Lung cancer is the second leading cause of death nationwide. 5-year survival improves dramatically (3.5% vs. 52.6%) for patients diagnosed while cancers are still localized. However, only 15% of patients are diagnosed at this early stage. Development of early stage biomarkers will drastically decrease death rates.

Stable isotope-resolved metabolomics (SIRM) provides a functional readout of cellular activity and is therefore a useful tool in biomarker discovery and fundamental cancer biology. We have established a primary lung squamous cell carcinoma line and studied it in situ and in mouse xenografts using SIRM. Here we report the metabolic activities of the cell line in culture.

Cells were grown with either [U–^{13}C]-glucose or [U–^{13}C,^{15}N]-glutamine and analyzed by ^{1}H-NMR, HSQC, TOCSY, GC-MS, and FTICR-MS. Cells exhibited high levels of glucose consumption that linearly correlated with lactate production. A strikingly high glucose-to-lactate conversion rate of 80% was observed. Glucose-derived carbon atoms were utilized in de novo biosynthesis of a broad range of metabolites, including the ribose subunit of nucleotides. Unique glutamine utilization and metabolite labeling patterns indicated that this cancer might be driven by C-MYC overexpression. C-MYC overexpression was confirmed using protein metabolomics-edited transcriptomics analysis (Pro-META). The ability of SIRM to generate hypotheses about protein expression demonstrates its potential use as a first line biomarker detection method. Comparison of cell culture data with upstream in situ and xenograft data will allow us to assess model validity and the effects of tumor microenvironment, and help us interpret human in situ data more reliably.

R25-CA-134283

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Title: Developing SOX9 Inhibitors [2013]

Authors: Danial Malik, B.A.,[1] John Trent, Ph.D, Mohammad Malik, Ph.D, Paula Bates, Ph.D. Biology[1] and Medicine and Biochemistry & Molecular Biology.[2]

Keywords: Cancer, SOX9, Virtual Screening, Drug Discovery

Abstract:

Cancer is the second leading cause of deaths in the United States. Current treatments lead to long-term survival in about two-thirds of people with cancer, but with increasing incidence rates for some cancers & an aging population the need for improved therapies remains. To maximize effectiveness while minimizing side effects, it is preferable to target molecules that are both specifically overexpressed in cancer cells & critical for cancer cell survival. The SOX9 transcription factor performs important functions during embryogenesis, but is rarely expressed in adult cells. Recent findings indicate that SOX9 expression is reactivated in many cancers & SOX9 has emerged as a master regulator of cancer stem cells (a subset of aggressive cancer cells that mediate recurrence & metastasis). Thus, small molecule inhibitors of SOX9 are exciting prospects as anticancer drugs. The purpose of this project was to evaluate compounds that had been identified as candidate SOX9 inhibitors by a virtual screening approach. A total of 182 compounds were selected for analysis at the time of modeling the crystal structure, SOX9 was not available. Following computational screening of 2.1 million compounds for their binding to defined sites within a homology model of SOX9 & their effects on the proliferation of SOX9-expressing cancer cells were determined using a colorimetric assay. Initial cell-based screening identified 10 compounds for further analysis & additional experiments were performed to assess cancer-selectivity & chemical stability. This allowed us to identify two compounds that show the most promising activity & selectivity for malignant vs. non-malignant cells. Supported in part by grant R25-CA-134283.

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Title: Inhibition of Glycolysis Using a Small Molecule Inhibitor PF158 in Combination with Temozolomide in Melanoma Cells [2013]

Authors: Adam Morrison,[1] Jason Chesney, PhD,[2] Julie O'Neal, PhD.[3] Arts & Sciences,[1] Medicine[2] and Brown Cancer Center.[3]

Keywords: Melanoma, Temozolomide

Abstract:

From 1979 to 2009 there has been an 800 percent increase in melanoma among young women and a 400 percent increase among young men[1]. It is estimated that 45,060 new cases of invasive melanoma in men and 31,630 in women will be diagnosed in the US in 2013[1]. Temozolomide (TMZ) is an alkylating agent commonly used in the treatment of melanoma. Response rates for metastatic melanoma remain poor, suggesting the need for alternative therapies. The mechanism that imparts TMZ with its therapeutic activity is its ability to methylate or alkylate DNA, which triggers apoptotic cell death. Glutathione (GSH) is involved in prevention and repair of DNA damage. Since we believe that the 6-phospho-2-kinase (PFK158) inhibitor decreases GSH, we hypothesized that the addition of PFK158 to cells treated with TMZ would increase cellular sensitivity to TMZ. PFK158 and TMZ were used in combination on the A375 melanoma cell line. The efficacy of the combination therapy was evaluated using flow cytometry to assess the death of the melanoma cells. We found that the combination of TMZ and PFK158 did cause additive cell death of A375 cells. This suggests the need for future investigation of this combination as a melanoma treatment.
This project was supported by grant R25- CA-134283 from the National Cancer Institute.

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Title: Sinusoidal endothelial cell-derived extracellular matrix regulates basal and stimulated macrophage activation [2013]

Authors: Lauren Poole, B.S.,[1] Jenny Jokinen, M.S. ,[1] Veronica Massey, M.S.,[1] Juliane Beier, Ph.D. ,[1] Gavin Arteel, Ph.D.[1] Pharmacology/Toxicology.[1]

Keywords: sinusoidal endothelial cells, hepatic inflammation, extracellular matrix

Abstract:

Fatty liver disease, be it from alcohol (ALD), obesity (NAFLD) or other sources (e.g. viral infection), involves chronic inflammation, although the mechanism(s) are unclear. One potential mechanism of chronic hepatic inflammation is crosstalk between the extracellular matrix (ECM) of hepatic sinusoidal endothelial cells (SEC) and resident macrophages. Here, this hypothesis was tested in vitro using cultured SECs and macrophages. Transformed hepatic sinusoidal endothelial cells (TSECs) were cultured for 72 hours. Culture plates were then washed with a solution that selectively removed the cells, but preserved the ECM. Cultured macrophages (RAW 264.7 cells) were seeded on the matrix and cultured for 24 hours; then stimulated with LPS for 0, 3, 6, 12, or 24 hours (10 or 100 ng/mL). Real time RT-PCR was used to measure mRNA expression of proinflammatory (IL-6, IL-1β, TNF-α, and INOS) and anti-inflammatory mediators (IL-10 and TGF-b). LPS stimulated production of all mediators by macrophages. With 100 ng/mL LPS, expression of IL-6, IL-1β, and IL-10 was attenuated by TSEC ECM, whereas expression of TNF-α, INOS, and TGF-β increased. Interestingly, TSEC ECM effect on the response to lower dose LPS (10 ng/ml) tended to be opposite to that observed with the higher dose. Experiments with the integrin inhibitor, CycloRGDfV, indicated some effects may be mediated via TSEC ECM binding to integrin receptors. These data serve as first proof-of-concept that macrophage activation can be modulated by TSEC-derived ECM and identifies a new interaction between these cells that may contribute to inflammatory liver disease. Grant support: R25- CA-134283 (NCI).

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Title: Candidate drugs binding the Anaphase Promoting Complex: A novel target for anti-cancer therapy [2013]

Authors: Douglas Saforo, BS,[1] Brian Sils, BS,[1] J. Christopher States, Ph.D.[1] Pharmacology and Toxicology.[1]

Keywords: Anaphase Promoting Complex, ANAPC2, APC/C, Apoptosis, Chemotherapeutic, Mitotic arrest, Paclitaxel resistance, Protein expression

Abstract:

Spindle poisons (e.g. paclitaxel) induce mitotic arrest and apoptosis in cancer cells. The anaphase promoting complex/cyclosome (APC/C) is an E3 ubiquitin ligase and the master regulator of cell cycle progression. Activation of the spindle assembly checkpoint (SAC) by spindle poisons inhibits the APC/C and induces mitotic arrest. Spindle poisons depend on functional SAC to induce mitotic arrest. Direct inhibition of the APC/C obviates the need for a functional SAC. Homology structure models for interacting APC/C subunits ANAPC11 and ANAPC2 were used for *in silico* screening of ANAPC2 to identify compounds predicted to interfere with ANAPC11 binding. Previous studies showed 3 compounds induced mitotic arrest and apoptosis in carcinoma cells but not in telomerase immortalized human fibroblasts. Thermal denaturation assay of recombinant protein can confirm on-target binding. Expressing recombinant 6XHis tagged ANAPC2 failed. The ANAPC2 cassette was then ligated into a new expression system utilizing a chitin-binding affinity tag and intein mediated purification. C-terminal (pTXB1-X10) and N-terminal (pTYB21-Y8) fusion constructs were prepared and tested in three expression hosts and evaluated for optimal yield. Large scale preparation of recombinant ANAPC2 using *E.coli* host BL21 expressing the N-terminal fusion protein is in progress. Compound binding to purified recombinant ANAPC2 will be determined. Compound binding of ANAPC2 will indicate that compounds targeting the APC/C can induce mitotic arrest and kill cancer cells while sparing normal cells and may be an effective approach to developing new anti-cancer drugs. Supported by National Cancer Institute grant R25-CA-134283.

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Title: Endocytic Trafficking of Mutant Epidermal Growth Factor Receptors in Lung Cancer [2013]

Authors: Tejas Sangoi,[1] Brian Ceresa, PhD. Arts & Sciences[1] and Pharmacology & Toxicology.[2]

Keywords: EGFR, Trafficking, Cancer, Lung

Abstract:

Purpose: To better understand the endocytic pathway and mechanism of how activating T790M mutants of epidermal growth factor receptor (EGFR) regulates signaling in two lung carcinoma cell lines: PC-9 and H1650.

Methods: PC-9 and H1650 cells express mutant EGFRs; HeLa cells express wild type EGFRs. Immunoblotting was used to test for time-dependent EGFR phosphorylation and degradation. Receptor trafficking and endosomal accumulation was observed by immunofluorescence staining of the EGFR. Kinetics of 125I-EGF endocytosis and ligand-mediated degradation was measured by radioligand binding assay.

Results: Immunoblotting shows that EGF treatment causes an increase in the basal activity of EGFR phosphorylation, and peaks at 30 minutes. Immunofluorescent staining of the EGFR indicates that a substantial amount of the unliganded, mutant EGFR accumulates in the early endosome and EGF treatment further increases endosomal accumulation in the mutant EGFR. Furthermore, there is a slower rate of receptor degradation of the mutant EGFRs compared to wild type receptor, as seen in the radioligand binding assay.

Conclusions: Activating mutants of EGFRs found in lung cancer cells not only have higher basal phosphorylation, but also defective endocytic trafficking. Based on radioligand binding and immunofluorescence experiments, it can be concluded that the receptor trafficking defect causes increased receptor accumulation in the endosome. Less degradation in the cancer cell lines indicates there is a disruption in the endocytic trafficking of mutant EGFRs.

Supported by grant R25-CA-134283 from the National Cancer Institute

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Title: Automated G-Quadruplex Generation: From Sequence To Structure [2013]

Authors: Nicholas Siow,[1] Yidi Huang,[2] Huy Le, B.S.,[3] Jon Maguire,[2] John Trent, Ph. D.[1] Medicine,[1] James Graham Brown Cancer Center[2] and Biochemistry and Molecular Biology.[3]

Keywords: quadruplex, automation, programming, g-tetrad, computational, g-quadruplex

Abstract:

In the search for targeted anti-cancer drugs, the choice of molecular targets is of fundamental importance. Tetrahelical structures known as quadruplexes are formed by sequences of guanine-rich DNA and are promising possible targets due to their highly diverse topologies offering a variety of target sites. Any changes in one of the G-quadruplex properties - such as number of G-tetrads in the quadruplex, glycosyl torsion angles, position in the sequence, G-tetrad stacking order, DNA directionality, or loop progression - will result in a structurally unique quadruplex. While computational models of these structures could be valuable resources, the degree of polymorphism within quadruplex-forming sequences makes manual production of these models a difficult task. Therefore, an automated means of quadruplex generation is a far more attractive solution for representing the full range of variation within quadruplexes. Such a solution would independently alter the quadruplex size, stacking order, glycosyl torsion angles, loop type, and loop progression to allow for the creation of each unique quadruplex.

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Title: In vivo and in vitro determinates that regulate BCLxl's apoptotic potency [2013]

Authors: Amy Song,[1] Levi Beverly, PhD.[2] Arts & Sciences[1] and Pharmacology and Toxicology.[2]

Keywords: BCLxL, BCL-2, apoptosis

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. Previous *in vivo* experiments in the lab using mouse models showed the significance of individual domains within BCLxL's protein structure for dictating oncogenic potency. Specifically, the Bcl-2 homology (BH)-4 domain of BCLxL has been shown to be essential in the anti-apoptotic functionality of BCLxL. *In vitro* experiments were performed to analyze how altering the protein structure of BCLxL affected protein stability. Cells were transfected with chimeric BCLxL proteins and then treated with either a proteasome inhibitor (MG132) or a protein biosynthesis inhibitor (cycloheximide). Data showed that cells treated with cycloheximide significantly decreased the expression of BCLxL, whereas cells treated with MG 132 had no change or an increase in levels of protein expression. In contrast, treatment of chimeric BCLxL protein expression increased dramatically within 4 hours of MG132 treatment, but by 16 hours post-treatment, levels had decreased and returned to levels seen pre-treatment. Previous research has shown certain residues in the BH4 domain of Bcl-2 to be significant to the potency of the protein. Attempts were made to induce point mutations in the BH4 domain of BCLxL through site-directed mutagenesis. The data obtained from these analyses will be used to standardize results of future experiments.

Research supported by grant R25-CA-134283 from the National Cancer Institute.

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Title: Comparing target protein levels between non-neoplastic and sporadic CRC tissue based on dysregulated miRNAs [2013]

Authors: Vanessa States,[1] Steven Girdler,[1] Jonathan Rice, MD.,[1] Henry Roberts, BS.,[1] Maurice Eichenberger, MS.,[1] Susan Galandiuk, MD.[1] Surgery.[1]

Keywords: colorectal cancer, miRNA

Abstract:

Introduction

Colorectal cancer (CRC) is the third most common cancer worldwide. Early detection is key in successful CRC treatment. Dysregulated miRNA expression is known to play a role in carcinogenesis. Protein targets of miRNAs are potential therapeutic modalities. Our previous results indicate miRNA expression in snap-frozen colon tissue significantly differs between non-neoplastic and cancerous samples.

Hypothesis

We hypothesize that miRNA expression in both snap-frozen and formalin fixed paraffin embedded (FFPE) CRC tissue is similar. Additionally, proteins targets of dysregulated miRNAs will have different expression levels in CRC as opposed to non-neoplastic tissues from the same patient.

Methods

Sections of non-neoplastic and cancerous tissue were removed from FFPE CRC slides by laser capture microdissection (LCM). miRNA was islolated and qRT-PCR was used to detect miRNA expression. In non-neoplastic and cancerous tissue, the levels of miR-31, miR-135b, miR-21, miR-1, and miR-133a were determined using TaqMan single assays. We utilized western blot analysis to determine relative expression of miRNA protein targets of miR-21 (PDCD4, PTEN, TGF-β) and miR-31 (RASA1).

Results

Similar miRNA expression levels were observed between FFPE and snap-frozen tissues, however protein extraction from FFPE tissue did not produce significant yield. Protein

expression levels were different between non-neoplastic and cancerous colon tissue.

Conclusion

Previous miRNA findings in snap-frozen CRC tissue have been validated in FFPE tissue.
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Public Link: http://ocrss.louisville.edu/clients/hscro/conspectus/searchview.php?ID=3527