



Annual Scientific Retreat

September 8, 2011

John Wright Forestry Center



Annual Scientific Retreat

September 8, 2011
8:00 am to 2:30 pm

[John S. Wright Forestry Center](#) ([map and directions](#))

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|--------------------------------------|--|
| 7:45-8:15 am | Continental Breakfast (Food will be available during the morning) |
| 8:15-8:30 am | Welcome and Overview of Direction: Tim Ratliff , Robert Wallace Miller
Director |
| 8:30-8:45 am | Deputy Director Update: Andrew Mesecar , Deputy Director |
| 8:45-9:00 am | Development Update: Tim Bobillo , Director of Development |
| 9:00-9:15 am | <u>Ulrike Dydak</u> , Assistant Professor of Health Sciences, College of Health
& Human Sciences
<i>"In Vivo 31P Magnetic Resonance Spectroscopy: Monitoring the
Liver's Response to Radiation Treatment"</i> |
| 9:15-9:20 am | Questions |
| 9:20-9:50 am | <u>Olaf Wiest</u> , Professor, Department of Chemistry & Biochemistry,
University of Notre Dame
<i>"Epigenetics in Cancer and Metabolic Diseases"</i> |
| 9:50-10:00 am | Questions |
| 10:00-10:20 am | Break |
| 10:20-10:35 am | <u>Keith Stantz</u> , Associate Professor of Health Sciences, College of Health
and Human Sciences
<i>"Photoacoustic Computed Tomography: Preclinical Imaging and
Image-Directed Therapy"</i> |
| 10:35-10:45 am | Questions |
| 10:45-11:00 am | Silvia Stan , Assistant Professor of Foods and Nutrition, College of Health
and Human Sciences
<i>"Cancer Prevention with Diet-Derived Compounds"</i> |
| 11:00-11:10 am | Questions |
| 11:10-11:15 am | Closing Remarks, Tim Ratliff |
| 11:15 am-12:00 pm
12:00 – 2:30 pm | Poster Session Setup and Lunch
Poster Session |



Poster Abstracts

Posters will be displayed in numerical sequence as listed below:

Poster #	PI/Faculty	Student Presenting Poster
1	Briggs, Scott	Kayla Harmeyer
2	Briggs, Scott	Paul South
3	Camarillo, Ignacio	Therese Salameh
4	Cushman, Mark	Evgeny Kiselev
5	Cushman, Mark	Abdelrahman Mayhoub
6	Dydak, Ulrike	Scott Jones
7	Fleet, James	Yan Li
8	Freeman, Jennifer	Greg Weber
9	Gelvin, Stanton	Lan-Ying Lee
10	Hall , Mark	Christie Eissler
11	Hu, Chang-Deng	Christopher Suarez
12	Hu, Chang-Deng	Chih-chao Hsu
13	Kim, Chang	Chuanwu Wang
14	Kirshner, Julia	Mukti Parikh
15	Kirshner, Julia	Mary Zheng
16	Konieczny, Stephen	David Hess
17	Liu , Xiaoqi	Shawn Liu
18	Liu, Xiaoqi	Bing Song
19	Liu, Shuang	Guoqiang Shao
20	Liu, Shuang	Yang Zhou
21	Mendrysa, Susan	Sara Ghassemifar
22	Mendrysa, Susan	Reem Malek
23	Stauffacher, Cynthia	Greg Costakes
24	Teegarden, Dorothy	Wei Zheng
25	V. Jo Davisson	Anthony Pedley

Understanding the Function of the Yeast Histone Demethylase Jhd2

Kayla M. Harmeyer, Paul F. South, and Scott D. Briggs, Department of Biochemistry Purdue University, Purdue Center for Cancer Research, Purdue University, West Lafayette, In 47907

Gene expression has been shown to be regulated through epigenetic modifications to the N- and C-terminal tail of histones. Among these modifications is methylation of lysine residues with the addition of one, two or three methyl groups (mono-, di- or trimethylation). The degree of methylation is not static. In *Saccharomyces cerevisiae* Jhd2 is the histone demethylase that is known to remove the methyl mark at histone H3 lysine 4 (H3K4), a mark commonly associated with active transcription. Our lab has shown that Jhd2 is regulated by the E3 ubiquitin ligase Not4, which targets Jhd2 to the proteasome for degradation. However, how Jhd2 interacts with the nucleosome to facilitate H3K4 demethylation is still unknown. It has been reported that when H3K14, a known acetylation site, is mutated H3K4 trimethylation levels are greatly reduced. *In vivo* experiments show that H3K4 trimethylation levels can be restored to wild type levels when H3K14 is mutated in a *jhd2* Δ strain. Through chromatin immunoprecipitation (ChIP) analysis we show that this is due to higher levels of Jhd2 protein associated with chromatin. Our lab has previously shown that Jhd2's PHD is necessary to see a reduction in H3K4 trimethylation when overexpressed. Furthermore, we identify an acid patch upstream from Jhd2 that is necessary to see a reduction if H3K4 trimethylation when Jhd2 is overexpressed. We also have evidence that this acid patch and PHD finger of Jhd2 interacts with H3K9 acetylation. Therefore, we propose a model that Jhd2 is interacting with the nucleosome depending on the acetylation state of the H3 N-terminal tail and that this interaction is mediated through the acid patch and/or PHD finger of Jhd2. Yeast Jhd2 is a homologue of the human JARID1 family of histone demethylases, which has four members: JARID1A, B, C and D. JARID1B is of particular interest because it has been shown to be up regulated in 90 percent of primary breast cancers and is closely associated with a malignant phenotype. JARID1B was also found to be up regulated in prostate cancer. Furthermore, JARID1A has been shown to be up regulated in gastric cancer. Using the yeast model system, we can gain valuable information on how H3K4 demethylases function, which in turn will allow us to better comprehend the role that H3K4 histone demethylases play in oncogenesis.

FUNCTIONAL CHARACTERIZATION OF THE DOMAINS OF HISTONE METHYLTRANSFERASE MEMBER BRE2 AND THE HUMAN HOMOLOG ASH2L

Paul F. South, Scott D. Briggs. Department of Biochemistry, Purdue Cancer Center, Purdue University, West Lafayette, IN 47907.

Epigenetic control through chromatin modification is important for regulating gene expression and proper cellular differentiation. One such chromatin modification is the methylation of Histone H3 lysine 4 (H3K4), which is commonly associated with active gene transcription and *HOX* gene regulation. Disruption of either the methyltransferases involved in H3K4 methylation, or *HOX* gene expression can lead to oncogenesis. In humans there are several H3K4 methyltransferases, all of which exist in a multi-subunit complex, including MLL 1-4 and Set1A and B. The human MLL and SET1 complexes share most of their core subunits and these are homologous to the subunits found in the yeast H3K4 methyltransferase complex Set1C. It has been unclear how individual Set1C members interact with each other and with its target chromatin, and how this interaction may impact histone methylation and gene expression. In this study we use yeast Bre2 the human homolog of the oncoprotein ASH2L to understand how this complex member interacts with the rest of the Set1 complex and with chromatin. We show that the conserved SDI domain within Bre2 and ASH2L are important for the interaction with its binding partner Sdc1 and the homolog hDPY-30 respectively. We also demonstrate that the SPRY domain within Bre2 is needed for interaction with chromatin independent of the rest of the histone methyltransferase complex. We begin to investigate the role of the PHD/Winged Helix motif region of Bre2 in complex binding and show that ASH2L interacts with the repeating subunit of chromatin (nucleosomes) independent of other human methyltransferase complex members. Overall, these and other mechanistic studies on how H3K4 methyltransferase complexes function will likely provide insights into how human MLL/SET1-like complexes or how overexpression of ASH2L leads to oncogenesis.

Palmitoleic Acid Levels Are Associated With Tumor Aggressiveness In Breast Cancer

Therese S. Salameh¹, Elbert L. Xu¹, Mateusz A. Stochliski², Camellia Reyes¹, Zhidong Xu³, John R. Burgess², Rafat A. Siddiqui³ & Ignacio G. Camarillo¹

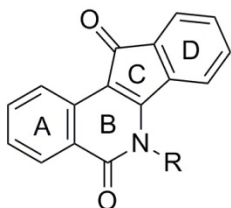
Department of Biological Sciences¹, Department of Foods & Nutrition², Purdue Cancer Center, Purdue University, West Lafayette, IN 47907. Cellular Biochemistry Lab³, Methodist Research Institute, Indianapolis IN 46202

De novo fatty acid synthesis is an important part of cancer progression as proliferating cancer cells need a continuous supply of lipids for membrane production. Synthesized fatty acids are also used for energy production through β -oxidation and lipid modification of proteins. It is thought that the *de novo* fatty acid synthesis pathway represents a viable target for cancer therapeutics. Our lab has recently developed an *ex vivo* adipose-tumor cell co-culture system for studying breast cancer, which has been verified by CARS microscopy to mimic the tumor microenvironment. This co-culture system enables the investigation of various aspects of tumor progression. For example, we have demonstrated adipose tissue alone is capable of stimulating growth and migration of the rat mammary epithelial tumor cell line CRL1743. The conditioned media from co-culture experiments induces CRL1743 cell proliferation, while the removal of fatty acids from conditioned media inhibits cell proliferation. Gas chromatography analysis of the fatty acids in the co-culture system components demonstrates, when tumor cells are present, there is an increase in fatty acids of the *de novo* synthesis pathway in tumor cells, conditioned media, and adipose tissue samples. The *de novo* fatty acids with the greatest change in levels were palmitoleic acid, linoleic acid, and oleic acid. Towards defining the role of these fatty acids, we show palmitoleic acid stimulates proliferation of CRL1743 cells and migration of MCF-7 human breast tumor epithelial cells. Furthermore, our preliminary results from fatty acid analysis of mammary fat tissue from Sprague-Dawley rats fed various diets (omega-3 rich, omega-6 rich, and western diet), indicates a correlation between tumor aggressiveness and palmitoleic acid levels. In summary, our work supports this co-culture model is an excellent intermediate between 2D *in vitro* cell culture studies and costly *in vivo* studies. The system provides a valuable means to study stromal-epithelium interactions and to develop potential cancer therapeutics targeting the *de novo* fatty acid synthesis pathway.

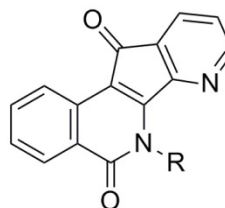
7-AZAINDENISOQUINOLINES: TOPOISOMERASE I INHIBITORS WITH IMPROVED WATER SOLUBILITY

Evgeny Kiselev,[†] Sean DeGuire,[†] Andrew Morrell,[†] Keli Agama,[‡] Thomas Dexheimer,[‡] Yves Pommier,[‡] and Mark Cushman.^{††} [†]Department of Medicinal Chemistry and Molecular Pharmacology, School of Pharmacy, and The Purdue Center for Cancer Research, Purdue University, West Lafayette, Indiana, 47907; [‡]Laboratory of Molecular Pharmacology, Center for Cancer Research, National Cancer Institute, Bethesda, MD 20892-4255.

A series of 7-azaindenoisoquinoline topoisomerase I (Top1) inhibitors have been prepared to investigate the effect of increased electron deficiency of the aromatic system on the ability to stabilize Top1-DNA cleavage complex. Ab initio calculations suggest that introduction of the nitrogen into the aromatic system of indenoisoquinolines would facilitate charge transfer complex formation with DNA, thus improving the π - π stacking interactions. The present study shows that 7-azaindenoisoquinolines demonstrate substantially improved water solubility without decrease in the Top1 inhibitory activity or cytotoxicity. Analysis of the results of biological evaluation revealed that some lactam ring substituents enable intercalation into free DNA whereas others only allow binding to the Top1-DNA cleavage complex.



An Indenoisoquinoline

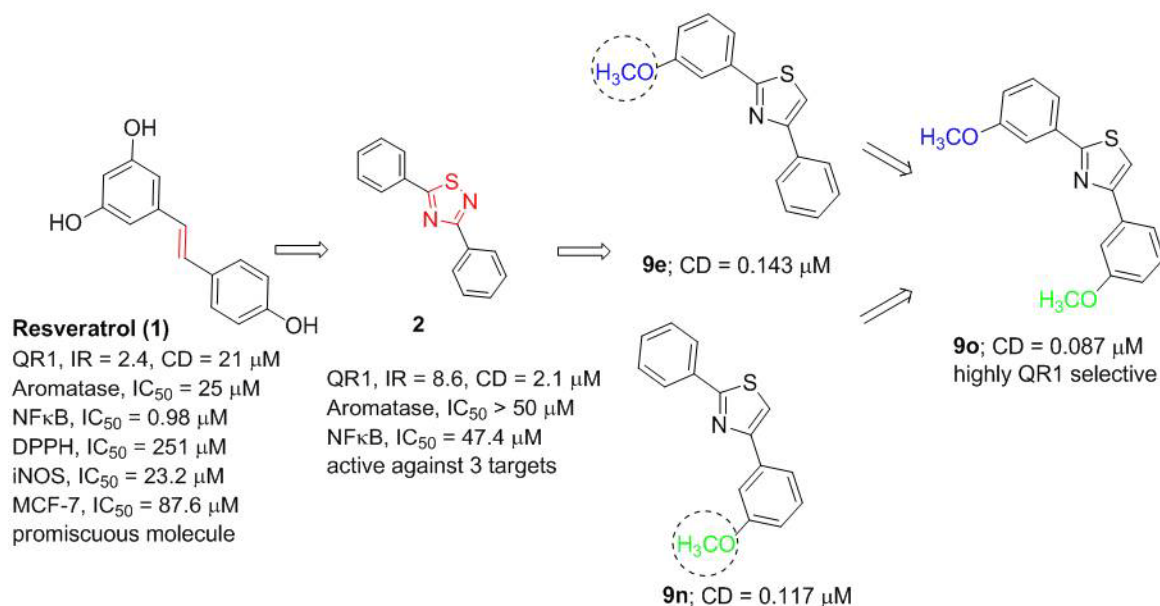


A 7-Azaindenoisoquinoline

OPTIMIZATION OF THIAZOLE ANALOGUES OF RESVERATROL FOR INDUCTION NAD(P)H:QUINONE REDUCTASE 1 (QR1)

Abdelrahman S. Mayhoub,[§] Laura Marler,[‡] Tamara Kondratyuk,[‡] Eun-Jung Park,[‡] John M. Pezzuto,[‡] and Mark Cushman^{§,*} [§]Department of Medicinal Chemistry and Molecular Pharmacology, College of Pharmacy, and the Purdue Center for Cancer Research, Purdue University, West Lafayette, Indiana 47907; [‡]College of Pharmacy, University of Hawaii at Hilo, Hilo, HI 96720

NAD(P)H:quinone reductase 1 (QR1) belongs to a class of enzymes called cytoprotective enzymes. It exhibits its cancer protective activity mainly by preventing formation of intracellular semiquinone radicals, and by generating α -tocopherolhydroquinone, which acts as a free radical scavenger. Therefore, it is believed that QR1 inducers can act as cancer chemopreventive agents. Resveratrol (**1**) is a naturally occurring stilbene derivative that requires a concentration of 21 μ M to double QR1 activity (CD = 21 μ M). The stilbene double bond of resveratrol was replaced with a thiazole ring and the phenols were eliminated to provide a more potent and selective derivative **2** (CD = 2.1 μ M). Optimizing the substituent pattern of the two phenyl rings and the central heterocycle linker led to a highly potent and selective QR1 inducer **9o** with a CD value of 0.087 μ M.



³¹P MRSI of Human Liver metabolism: Bridging the Gap between Therapy and Diagnostic Imaging

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Department of Radiology and Imaging Sciences, Indiana University School of Medicine, Indianapolis, IN

Phosphorous magnetic resonance spectroscopic imaging (³¹P MRSI) is a non-invasive diagnostic tool that has demonstrated clinical potential in assessing energy and phospholipid metabolism in healthy and diseased human liver. Despite multiple clinical research studies correlating ³¹P liver metabolite levels (ATP, Pi, PME, PDE) with acute injury (e.g. hepatectomy) and chronic disease (e.g. cancer, cirrhosis, etc.), the clinical role of ³¹P MRSI for evaluating the metabolic profile of healthy and diseased liver has frequently been hampered by technical and physical limitations and largely resulted in non-translational clinical applications. Therefore, the goals of this research were to: (1) optimize clinical ³¹P MRSI protocols in healthy subjects using a novel dual-tuned ¹H/³¹P 8-channel phased array liver coil specifically designed to acquire large field of view (FOV) in-vivo ³¹P spectroscopic imaging on a commercial whole body 3.0T MRI scanner, (2) acquire ³¹P MRSI data from human subjects with hepatocellular cancer prior to, 24-72 hours after, and 4-6 weeks after radiation treatment, and (3) evaluate the diagnostic and/or prognostic value of spatially resolved radiation induced metabolic alterations in both healthy and malignant liver tissue. Spectroscopic imaging results have shown the ability of our novel ³¹P MRS liver coil to acquire ³¹P spectroscopic imaging of healthy liver throughout a full axial slice or whole liver volume. Furthermore, the data quality collected from both the axial slice and whole liver volume enabled us to map the spatial distribution of ³¹P metabolites throughout the whole liver volume, which to the best of our knowledge, has not been done prior to this study. Preliminary clinical results have demonstrated that in addition to its ability to differentiate the metabolic signatures of malignant and non-malignant tissue in the pre-treatment analysis, ³¹P MRSI has also detected metabolic changes at 4-6 weeks post treatment that indicated a positive treatment response. Treatment response, in all cases, was confirmed 6 months post treatment by CT imaging and RECIST criteria. In conclusion, we present a novel dual-tuned ¹H/³¹P 8-channel phased array liver coil and clinically optimized protocol capable of acquiring ³¹P MRSI data from the whole liver volume. Preliminary data indicates that ³¹P MRSI has clinical potential, but more research is still needed.

*both authors contributed in equal amount

GENE EXPRESSION PROFILING OF TgAPT₁₂₁ MOUSE PROSTATE REVEALS BIOLOGICAL PROCESSES ASSOCIATED WITH EARLY PROSTATE CARCINOGENESIS

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The TgAPT₁₂₁ transgenic mice express a truncated SV40 T antigen (T₁₂₁) in prostate epithelial cells and have been proposed as a model for the early stages of prostate cancer (PCa) development. T₁₂₁ expression inactivates the retinoblastoma family of proteins, causing epithelial hyperplasia, prostatic intraepithelial neoplasia (PIN) by 12-wk of age, and microinvasive adenocarcinoma by 6-mo of age. Here we examined how the molecular features of the anterior prostate (AP) in this model relate to its histological presentation of PIN. TgAPT₁₂₁ and non-transgenic control mice were raised to 12 wks, at which time the AP were removed and processed for histology and RNA isolation. Transcripts were analyzed using the Affymetrix Mouse Gene 1.0 ST array. Data were preprocessed using the Bioconductor packages; differentially expressed genes were identified by SAM, and functional analysis was conducted using the MetaCore analysis suite (GeneGo, Inc.) More than 90% of the prostate epithelium developed PIN lesions and 4271 transcripts were significantly altered (FDR < 5%, fold-change > 1.5; 3745 unique genes, 2433 up, 1312 down). A published microarray dataset on androgen-stimulated prostate growth (GSE5901) was used to identify genes controlling proliferation. Proliferation-related genes were removed from the TgAPT₁₂₁ dataset in order to identify hyperproliferation-independent genes associated with PIN development. Functional analysis of this subset of genes revealed enrichment of pathways related to DNA damage, apoptosis, cytoskeleton remodeling, cell signaling, and cell metabolism pathways. Moreover, gene networks built around transcription factors known to be involved in human PCa were identified. For example, a p53 signaling network was identified as upregulated in our data and immunohistochemistry confirmed the accumulation of p53 protein in the epithelial cells of TgAPT₁₂₁ mice. In addition, functional analysis revealed enrichment of DNA damage repair mechanisms suggesting that p53 upregulation is an adaption in PIN lesions to suppress progression of PCa through this mechanism. Our data also showed differential expression of apoptosis-related genes and enrichment of apoptotic pathways, which is consistent with the increased apoptosis rate we observed by TUNEL staining. Upregulation of apoptosis is mediated by mechanisms in addition to p53, such as the TNF receptor family signaling. Signaling through these pathways may account for the p53-independent apoptosis previously reported in this model.

Genetic pathways underlying the immediate and lifespan impacts of a developmental exposure to the endocrine disrupting herbicide atrazine

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There is a growing body of evidence which indicates continual exposure to endocrine disruptors over the lifespan of an individual or during increased sensitivity windows (such as development) substantially elevates the risk to develop multiple types of cancers, including breast, ovarian, thyroid and prostate cancer. Atrazine, an herbicide commonly applied to agricultural areas throughout the Midwest and a common contaminant of potable water supplies, has been implicated as an endocrine disruptor and potential carcinogen. The specific adverse health effects associated with atrazine exposure and the underlying molecular mechanisms of these effects are not well defined. Several described endocrine disruptors often act as mimetics of ligands or hormones and competitively bind to targeted receptors such as the estrogen, androgen, dopamine or serotonin receptors and subsequently enhance or suppress the corresponding pathway(s). However, to date the molecular mechanism through which atrazine exerts its effects is not well understood. Thus, the objectives of this study aim to [1] first use microarrays to identify global gene expression alterations following embryonic exposure to atrazine and anchor these 'genetic signatures' to phenotypic responses, [2] identify lasting impacts of the atrazine developmental exposure on reproduction and disease-associated effects in adults by evaluating gene and protein expression alterations and reproductive success, and [3] identify epigenetic alterations in a subsequent generation. Initial genomic analysis immediately following the embryonic atrazine exposure has identified enrichment of genes with altered expression patterns that are involved in neuroendocrine development and disease. A subset of individuals was permitted to mature under normal conditions and lifespan and epigenetic effects of the developmental atrazine exposure are being assessed in sexually mature adults and in their offspring. A significant difference in the number of pairs that bred were observed, although there was no difference in sex ratio, average number of offspring per breeding pair, offspring mortality, or embryo hatching success. Moreover, a distinct altered reproductive morphological phenotype was observed in ~10% of female adult zebrafish developmentally exposed to atrazine indicating a persistent lifespan effect of the developmental atrazine exposure. In addition, multiple fish developmentally exposed to atrazine have presented with melanoma. The reproductive alteration in a subset of the females and the presence of melanoma provide further support to the endocrine disrupting effects of this herbicide and a potential role in carcinogenesis that warrant further investigation. Considering our genomic and phenotypic data, a subset of genes with prominent roles in reproductive function and cancer (including *Luteinizing hormone (LH)*, *CYP17A1*, and *GLO1*) has been chosen for targeted analysis in a subset of adult individuals developmentally exposed to atrazine and then allowed to mature under normal conditions and in their subsequent offspring (F₁ generation for epigenetic alterations).

PEPTIDE APTAMER 'MUTAGENESIS' FOR GENERATING PHENOTYPES IN CELLS

Lan-Ying Lee, Soyon Park, Joerg Spantzel, Yaling Wang, and Stanton B. Gelvin

Department of Biological Sciences, Purdue University, West Lafayette, IN 47907-1392

Peptide aptamers (from the Latin *aptus* for “fitting”) are short peptides of random sequence that can interact with specific target proteins *in vivo*. As commonly used, these peptides are generally 15-20 amino acids-long. This length provides enough flexibility for the peptide to assume various conformations while reducing the probability of randomly creating a stop codon in the aptamer coding sequence. We have developed peptide aptamer ‘mutagenesis’ technology for use in animals and plants. As proof of concept, we have targeted *Agrobacterium* VirE2 protein with various 20-mers from the VirE2 protein sequence. VirE2 is known to interact with several *Agrobacterium* and host plant proteins; for many of these protein pairs, sites important for interaction with VirE2 are known. In addition, a recent crystallographic study has indicated the structure of VirE2 in a complex with its bacterial chaperone VirE1. We have designed an aptamer expression cassette which uses bimolecular fluorescence complementation (BiFC) to detect aptamer-protein interactions. The cassette consists of a strong promoter + full-length mCherry + a multiple cloning site for insertion of aptamer coding sequences + nVenus + polyA addition signal. Expression of the aptamer cassette results in red mCherry fluorescence, indicating cells containing and expressing the aptamer. We have individually tagged VirE2 with cCFP and nCerulean. Dimerization of VirE2 in tobacco BY-2 protoplasts brings together cCFP and nCerulean, generating blue fluorescence. Interaction of VirE2-nCerulean with several of the tested mCherry-aptamer-nVenus polyprotein constructions generates yellow fluorescence, thus validating the use of BiFC as a detection system for protein-aptamer interactions. Interaction of VirE2 with aptamer #6 is especially strong in plant cells. Interaction of an aptamer with VirE2 may inhibit VirE2 function, resulting in decreased transformation of plant cells expressing the aptamer. We have generated hundreds of transgenic *Arabidopsis* plants that express various aptamers targeted to VirE2. Expression of aptamer #6 inhibits transformation using a standard root transformation protocol.

To facilitate the use of peptide aptamers to generate phenotypes, we have generated a library of 2×10^8 random aptamers in a Gateway donor vector. We have exchanged these aptamers into Gateway aptamer expression destination vectors. These expression libraries are designed to express aptamers in plant (using the CaMV 35S promoter) or animal (using the CMV promoter) cells. These libraries are available for the Purdue research community to use.

Cdc14 Phosphatases Selectively Dephosphorylate Cdk Sites Containing Phosphoserine

Steven Bremmer, Christie Eissler, Juan Martinez, Thomas Hinrichsen, Sandra Rossie, Laurie Parker, Mark Hall, and Harry Charbonneau

Mis-regulation of the proteins comprising the core cell cycle machinery can cause genome instability, often leading to cancer. Cyclin-dependent kinases (Cdks) are an essential component of the cell cycle control system and their activity is required to initiate cell division and drive a cell into mitosis. Cdk inactivation after the metaphase-anaphase transition is required for mitotic exit and cytokinesis. In addition, Cdk phosphorylation events must be reversed by protein phosphatases. In budding yeast, reversal of Cdk-dependent protein phosphorylation by the dual specificity phosphatase Cdc14 is essential for mitotic exit. However, very little is known about the cell cycle functions of the human Cdc14 orthologs, in part because few substrates are known. Genetic and limited biochemical data have strongly suggested that Cdc14 enzymes are generally specific for Cdk-type phosphorylation sites (Ser-Pro, or Thr-Pro, often followed by basic amino acids). However, we recently characterized the enzymatic specificity of several Cdc14 phosphatases and have found that they have an unusual and strict specificity for phosphoserine over phosphothreonine within Cdk phosphorylation sites. Additionally, Cdc14 has a strict requirement for a basic residue in the +3 position relative to the phosphorylated serine. The strict consensus recognition sequence for Cdc14 enzymes represents a useful tool for identifying new Cdc14 substrates. Our findings suggest that Cdc14 is responsible for reversing only a subset of Cdk phosphorylation events and therefore could contribute to the timing of Cdk substrate dephosphorylation that is important for ensuring fidelity of late mitotic events and genome stability.

Targeting CREB signaling as a strategy for prostate cancer radiotherapy

Christopher Suarez, Xuehong Deng, and Chang-Deng Hu

Department of Medicinal Chemistry and Molecular Pharmacology

Purdue Cancer Center, Purdue University, West Lafayette, IN

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

CRITICAL ROLE OF AN N-TERMINAL END-LOCALIZED NUCLEAR EXPORT SIGNAL IN REGULATION OF ACTIVATING TRANSCRIPTION FACTOR 2 (ATF2) SUBCELLULAR LOCALIZATION AND TRANSCRIPTIONAL ACTIVITY

Chih-Chao Hsu and Chang-Deng Hu. Department of Medicinal Chemistry and Molecular Pharmacology, Purdue Cancer Center, Purdue University, West Lafayette, IN 47907.

Activating transcription factor 2 (ATF2) belongs to the basic region leucine zipper (bZIP) family of transcription factors. ATF2 regulates target gene expression by binding to the cyclic AMP response element (CRE) as a homodimer or a heterodimer with c-Jun. Cytoplasmic localization of ATF2 was observed in melanoma, brain tissue from patients with Alzheimer's disease, prostate cancer specimens and ionizing radiation-treated prostate cancer cells, suggesting that alteration of ATF2 subcellular localization may be involved in the pathogenesis of these diseases. We previously demonstrated that ATF2 is a nucleocytoplasmic shuttling protein and it contains two nuclear localization signals (NLS) in the basic region and one nuclear export signal (NES) in the leucine zipper domain (named LZ-NES). In the present study, we demonstrate that a hydrophobic stretch in the N terminus, ¹MKFKLHV⁷, also functions as an NES (termed N-NES) in a chromosome region maintenance 1 (CRM1)-dependent manner. Mutation of both N-NES and LZ-NES resulted in a complete nuclear localization whereas mutation of the individual NES only partially increases the nuclear localization. These results suggest that cytoplasmic localization of ATF2 requires function of at least one of the NESs. Further, mutation of N-NES enhanced the transcriptional activity of ATF2, suggesting that the novel NES negatively regulates the transcription potential of ATF2. Thus, ATF2 subcellular localization is likely modulated by multiple mechanisms, and further understanding of the regulation of ATF2 subcellular localization under various pathological conditions will provide insight into the pathophysiological role of ATF2 in human diseases.

Origin of tumor-associated FoxP3⁺ T cells

Chuanwu Wang, Jeeho Lee and Chang H. Kim

Laboratory of Immunology and Hematopoiesis, Department of Pathobiology; Purdue Cancer Center; Bindley Bioscience Center; Purdue University, West Lafayette, IN 47907

Abstract

FoxP3⁺ regulatory T cells are thought to play both positive and negative roles in tumorigenesis and anti-tumor-immunity. The FoxP3⁺ T cell population is highly enriched in tumors but their origin and fate are still unclear. We investigated the frequencies, origin (thymus-generated versus induced), and migration of tumor-associated FoxP3⁺ T cells. We examined also de-differentiation of tumor-associated FoxP3⁺ T cells into FoxP3⁻ T cells. We report that only certain type of memory, but not naïve, FoxP3⁺ T cells are highly enriched in tumors. The data indicate that certain trafficking receptors play critical roles in regulation of migration and retention of FoxP3⁺ T cells within tumors. Importantly, antigen priming events are required for proper expression of the trafficking receptors by FoxP3⁺ T cells. Most FoxP3⁺ T cells are thymus-generated, but not induced, FoxP3⁺ T cells. The turnover rate of FoxP3⁺ T cells into FoxP3⁻ T cells is low in tumors. Taken together, our results revealed that tumor-associated FoxP3⁺ T cells are mainly originated from the thymus but not induced in secondary lymphoid tissues, and migration of these FoxP3⁺ T cells following appropriate antigen priming in secondary lymphoid tissues is critical for population of FoxP3⁺ T cells in tumors.

Seeding the Metastasis: Role of Cancer Stem Cells in Breast Cancer Spread to the Bone

Mukti Parikh^{1,2} and Julia Kirshner²

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Abstract:

Breast cancer is the most common malignancy in women, with 80% breast cancer patients suffering from invasive tumors. Bone is one of the most common sites of breast cancer spread and accounts for significant morbidity and mortality in patients. Dissemination is a very early event facilitating intravasation of breast tumor cells and hence a prerequisite for initiating distant site metastasis. Cancer stem cells (CSCs) have been implicated to be capable of establishing new tumors at secondary sites after dissemination. CSCs being highly drug resistant cannot be eliminated by current conventional treatments successful in removing the primary tumor. The first objective of this study is to evaluate the role of breast CSCs (B-CSCs) in metastatic spread of breast cancer to the bone. Using MCF10A series of human breast cancer progression cell lines in a 3-dimensional 'reconstructed metastasis (rMet) model' we plan to study presence of self-renewal potential and B-CSC markers in disseminated cells. Successful establishment of a metastatic lesion requires reattachment of breast tumor cells at a new site (bone) after extravasation. Detached breast tumor cells express a protein called Tau that promotes cytoskeletal rearrangements necessary for reattachment of disseminating cells. Therefore, another objective of this study is to elucidate the role of Tau in bone colonization by breast tumor cells and pinpoint particular isoforms that may be important for tumor cell dissemination, invasion and migration. Prevention of bone colonization in the rMet model by suppressing the identified Tau isoforms in metastatic cells will highlight the potential of Tau to be investigated for preventing metastatic breast cancer.

The Nature's Pharmacy: Development of Novel Therapeutic Agents Against Brain Metastases of Breast Tumors

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Breast cancer is the most commonly diagnosed cancer in women (excluding skin cancer) and is the second most deadly malignancy after lung cancer. One in eight American women will develop invasive breast cancer during their lifetimes. While survival rates for women with breast cancer have been steadily improving over the last decade, metastatic disease is still the leading cause of death from breast cancer, with 5-year survival rates for women with metastatic disease remaining below 20%. Bone, lung, brain, and liver are the most common sites of secondary spread of breast cancer. Treatment of metastatic disease has presented a major challenge, with brain metastases being especially difficult to treat. Brain metastases arise from highly aggressive disease and often afflict younger patients with Her2 positive primary tumors. Due to their localization to the privileged site, little progress has been made to design novel therapeutic agents against these metastatic lesions. A successful therapeutic compound would have to exhibit a number of unique characteristics, i.e. 1) ability to cross the blood-brain barrier, 2) specificity for the tumor cells, and 3) minimal neuronal toxicity. Here we propose to investigate a plant derived natural product, eburnamonine (EBN), as a therapeutic agent against brain metastases of breast tumors. EBN is a pharmacologically active compound derived from the flowering plant *Periwinkle* and exhibits vasoregulatory and anti-hypoxic properties. It has also demonstrated to cross the blood-brain-barrier and has been tested as a treatment for a number of cerebrovascular disorders, including ischemia and anoxia. EBN has no known neurotoxicity, in fact it has been found to be neuroprotective with no reported side effects. Thus, EBN meets all the criteria for a potential agent to combat brain metastasis from breast cancer and other tumors presenting with brain lesions. This project is designed to test EBN as a therapeutic agent to combat brain metastatic lesions from breast cancer and to define its mechanism of action.

ACINAR-SPECIFIC XBP1 ABLATION TRIGGERS APOPTOSIS AND COORDINATE ACINAR/CENTROACINAR REGENERATION IN THE ADULT MURINE PANCREAS

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Stress response pathways regulate cell survival and growth and are ideal candidates for therapeutic manipulation in treating pancreatic diseases. Our work sought to determine whether disruption of the IRE1/Xbp1 axis of the unfolded protein response (UPR) could result in targeted cell death specifically in pancreatic acinar cells. These cells are under heavy secretory load and utilize the UPR to respond to the accumulation of misfolded proteins, commonly referred to as ER stress. Acinar cells have also been implicated as the cells of origin in multiple mouse pancreatic cancer models, making an understanding of how to control their viability a possible first step in designing new therapeutics for pancreatic disease. To study this pathway's role in acinar cell homeostasis, Cre-inducible Xbp1 ablation was used while pancreata were monitored over a 12 week period. Pancreas morphology, protein expression, and cell viability were investigated via protein blot, immunohistochemistry, and electron microscopy, and gene expression analyses were conducted using RT-qPCR.

Xbp1 recombination was confirmed in ~90% of pancreatic acinar cells and resulted in ER stress accompanied by autophagy and apoptosis approximately four weeks post-ablation. Unrecombined acinar cells, as well as a subset of Sox9 and Hes-1 positive centroacinar cells, exhibited a robust proliferative response, regenerating the damaged exocrine tissue. Pancreatic protein and gene expression returned to near-wildtype levels following regeneration despite the appearance of enlarged acinar cells and Sox9-positive tubular complexes. We conclude that ablation of Xbp1 specifically in pancreatic acinar cells results in uncontrollable ER stress leading to eventual apoptosis. This damage triggers an acinar/centroacinar regenerative response that restores the organ, demonstrating that ER stress-induced cell death is well tolerated by the pancreas. Current and future investigations are focused on suppressing Xbp1 in the context of activated Kras expression as a means of controlling the viability of transformed cells.

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

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

Plk1 phosphorylation of Orc2 promotes DNA replication under conditions of stress

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Abstract

Polo-like kinase 1 (Plk1) plays pivotal roles in mitosis, however, little is known about its function in S phase. In this study, we show that inhibition of Plk1 impairs DNA replication and results in slow S-phase progression in cultured cancer cells. We have identified Origin Recognition Complex 2 (Orc2), a member of the DNA replication machinery, as a Plk1 substrate and have shown that Plk1 phosphorylates Orc2 at Ser188 *in vitro* and *in vivo*. Furthermore, Orc2-S188 phosphorylation is enhanced when DNA replication is under challenge induced by UV, hydroxyurea, gemcitabine, or aphidicolin treatment; cells expressing the unphosphorylatable mutant (S188A) of Orc2 had defects in DNA synthesis under stress, suggesting that this phosphorylation event is critical to maintain DNA replication under stress. To dissect the mechanism pertinent to this observation, we showed that Orc2-S188 phosphorylation associates with DNA replication origin and that cells expressing Orc2-S188A mutant fail to maintain the functional pre-replicative complex (pre-RC) under DNA replication stress. Furthermore, the intra-S phase checkpoint is activated in Orc2-S188A-expressing cells to cause delay of S-phase progress. Our study suggests a novel role of Plk1 in facilitating DNA replication under conditions of stress to maintain genomic integrity.

**Imaging Glioma and Recurrent Glioma by U-SPECT-II/CT
with ^{99m}Tc -3P-RGD₂, an Integrin $\alpha_v\beta_3$ -Targeted SPECT Radiotracer**

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Glioblastoma multiforme (GBM) accounts for 52% of all parenchymal brain tumor cases. Its overall five-year survival rate is approximately 5% because of delayed diagnosis, therapy and local recurrence due to diffuse infiltration resulting from angiogenesis. Integrin $\alpha_v\beta_3$, a receptor for the extracellular matrix proteins with the exposed arginine-glycine-aspartic (RGD) tripeptide sequence, plays a significant role in tumor angiogenesis and facilitates cellular adhesion and migration to extracellular matrix proteins. It is expressed at high levels on activated endothelial cells of tumor neovasculature and glioblastoma cells but at low levels on mature endothelial cells. ^{99m}Tc -3P-RGD₂ is a ^{99m}Tc -labeled cyclic RGD peptide, and binds to the $\alpha_v\beta_3$ with high specificity and selectivity. It is currently under clinical evaluations for diagnosis of carcinomas of breast, lung and prostate. In this study, we evaluated ^{99m}Tc -3P-RGD₂ for its capability to detect the glioma and recurrent glioma tumors in glioma-bearing athymic nude mice. The main objective of these studies is to demonstrate that ^{99m}Tc -3P-RGD₂ is useful imaging tool for SPECT-guided anti- $\alpha_v\beta_3$ therapy. We established both primary and recurrent glioma-bearing animal models in our laboratories. In the primary tumor model, glioma tumors could be clearly visualized by SPECT/CT on the 5th day post-injection of glioma cells subcutaneously. At this time, the tumor size was only $\sim 0.002\text{ cm}^3$ (or $\sim 2\text{ mg}$) and these tumors were not palpable. We also found that there was a direct linear relationship ($R^2 = 0.7735$) between the %ID tumor uptake (total tumor radioactivity) of ^{99m}Tc -3P-RGD₂ and the tumor size. However, the %ID/g tumor uptake (radioactivity density) peaked when the tumor volume was $0.15 - 0.25\text{ cm}^3$ (or $150 - 250\text{ mg}$). The tumor $\alpha_v\beta_3$ expression level was determined by immunohistochemical staining, and was correlated with the %ID/g tumor uptake (radioactivity density). In the recurrent glioma tumor model, glioma tumors were readily detectable even before they were not palpable. It was concluded that quantitative U-SPECT-II/CT imaging with ^{99m}Tc -3P-RGD₂ is particularly useful for both early detection of primary and recurrent glioblastoma and for noninvasive determination of the tumor $\alpha_v\beta_3$ expression level changes before and after therapy.

IMAGING BREAST CANCER METASTASIS BY U-SPECT/CT WITH $Tc-99m-3P-RGD_2$

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Breast cancer is the second leading cause of deaths among women worldwide. Metastatic breast cancer is characterized by spreading of cancer cells into nearby breast tissue, and other body parts (bone, lymph nodes, liver and lungs). During metastasis, breast tumor cells attach to extracellular matrix proteins, release proteases that degrade the basement membrane, and invade through degraded basement membrane to spread to other body parts through circulation. It has been reported that integrin $\alpha_v\beta_3$ plays a significant role in progression of invasive breast cancer. The integrin $\alpha_v\beta_3$ activation is required for hematogenous breast cancer metastasis. Integrin $\alpha_v\beta_3$ is an important prognostic biomarker for highly invasive breast tumors.

$^{99m}Tc-3P-RGD_2$ is a new radiotracer specific for the integrin $\alpha_v\beta_3$ overexpressed on breast cancer cells and tumor neovasculature. Our previous studies showed that $^{99m}Tc-3P-RGD_2$ is useful for early-detection of integrin $\alpha_v\beta_3$ -positive tumors in the xenografted animal models. To further expand its diagnostic utility, we established two breast cancer metastasis models, and used a u-SPECT/CT scanner to image breast cancer metastases with $^{99m}Tc-3P-RGD_2$ as the radiotracer. Our imaging data clearly showed that breast cancer metastasis lesions in lungs, mandible, maxilla, spinal cord, rib, neck and lymph nodes in the underarms could be readily detected. Histological staining studies further confirmed the existence of most tumors. The smallest lesion detectable by u-SPECT/CT is ~1 mm in diameter. Larger breast tumor tissues (>0.2 g) were quite heterogeneous with respect to the radiotracer uptake and integrin $\alpha_v\beta_3$ expression levels due to the presence of necrosis. In necrotic area of the tumor tissue, there was very low integrin $\alpha_v\beta_3$ expression, leading to low uptake of $^{99m}Tc-3P-RGD_2$. Our imaging data clearly showed that each animal had its own distinctive metastasis patterns. $^{99m}Tc-3P-RGD_2$ is an excellent radiotracer for detection of breast cancer metastasis to bone and lungs.

TARGETING THE MDM2-P53 AXIS IN MEDULLOBLASTOMA USING NUTLIN-3

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Medulloblastoma (MB), a cerebellar tumor, is the most common malignant brain tumor in children. One subtype of MB is thought to arise from a Granular Cell Precursor (GCP) during cerebellar development through the deregulation of the normal signaling pathways that control GCP proliferation, survival and differentiation. The prognosis of MB tumor is poor as current treatments lead to long-term side effects especially in infants and younger children. New therapies that improve the killing of tumor cells while sparing normal cells are critically needed. One potential strategy to improve the efficacy of currently used chemotherapeutic agents especially in tumors with wild type p53 is to enhance p53 activity by disrupting the interaction of p53 with its principal inhibitor, MDM2. Our previous studies indicate that MDM2 is required for MB formation as a 70% reduction in the level of MDM2 abrogates the formation of preneoplastic lesions in the mouse model of human MB which provides the rationale for the use of MDM2 inhibitors in MB treatment. Using small molecules such as Nutlin-3 that inhibit the interaction of MDM2 and p53 is the center of attention in many cancer therapies. We predict that Nutlin-3 would be beneficial in treating MB as p53 is wild-type in most of these tumors. Moreover, Nutlin-3 is known to be less toxic to normal cells. Thus, it is anticipated that treatment with Nutlin-3 leads to less side effects in MB patients. In this study, the physiological outcome of enhanced p53 function was investigated in a panel of human MB cell lines. We present evidence that in p53 wild-type MB tumor cells, Nutlin-3 promotes apoptosis and/or cell cycle arrest and activates p53 synergistically with doxorubicin. In contrast, p53 mutant MB cell lines are highly resistant to Nutlin-3 treatment. However, at high concentrations, Nutlin-3 could induce also apoptosis in p53 mutant MB cell lines suggesting that Nutlin-3 may also be effective in p53 mutant MB tumors through an alternative mechanism. Our data suggest that Nutlin-3, in combination with other chemotherapy drugs, may improve the treatment of patients with MB.

MDM2-P53 INTERACTION DRIVES CEREBELLUM DEVELOPMENT AND MEDULLOBLASTOMA FORMATION

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During cerebellar development, the Granule Neuron Precursors (GNPs) undergo a proliferative expansion followed by differentiation and migration to their final niche. The process is tightly regulated and any anomaly can tip the scale toward aberrant proliferation leading to Medulloblastoma (MB) or cell cycle arrest, apoptosis and abnormal differentiation, leading to neurodegenerative diseases. We report that Mdm2, the principal inhibitor of the tumor suppressor p53, is required for normal development of the cerebellum as well as MB tumor formation.

Here, we made use of Mdm2 hypomorphic mice (*Mdm2^{puro/Δ7-9}*) that express only ~30% Mdm2 and show increased p53 activity in the cerebellum. The gross cerebellar morphology of these mice shows a decrease in foliation, reduction in GNP numbers and disrupted laminar organization. Fate mapping experiments point to premature GNP differentiation and migration defects. Further, microarray analysis on whole cerebellum suggests dysregulation of the genes upregulated during GNP differentiation. In agreement, we observe an increase in the prodifferentiation protein, Numb and the marker of mature neurons, βIII-Tubulin) in purified preparations of GNPs. A concomitant proliferation defect is indicated by attenuation in the Shh (Sonic hedgehog) signaling and a decrease its effectors Gli1 and Gli2, which drive GNP proliferation. These experiments suggest that loss of Mdm2 and subsequent increase in p53 activity favors differentiation over proliferation of GNPs .

In complementary experiments, we made use of primary GNP cultures from wild type mice that can proliferate in response to Shh. Shh stimulation of these cultures results in an increase in Mdm2 and its phosphorylation at Ser 166, a modification known to have enhanced p53 degrading capability. This suggests that Shh signaling may upregulate Mdm2 to prevent p53 activation during the proliferative expansion of GNPs. In keeping with the conserved mechanisms of GNP proliferation and MB tumor formation, we also observed increased levels of P-Mdm2^{Ser166} in Shh induced MB tumors and preneoplastic lesions in a mouse model of MB (*Ptch^{+/-}*). Notably, reduction of Mdm2 levels in these mice abrogates the formation of PNL formation in a dose dependent fashion, indicating that Mdm2 may play a key role in tumorigenesis in this subtype of MB.

Overall, our results suggest that Mdm2-p53 loop may play a key role in balancing Shh induced proliferation and the subsequent differentiation of the GNPs. This has important implications for GNP differentiation defects as well as the Shh subtype of MB tumors.

***In-vitro* and *in-cell* Characterization of Human Cytoplasmic Protein Tyrosine Phosphatase Variants**

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Arising from the ACP1*B allele of human acid phosphatase 1, the electrophoretically different human cytoplasmic protein tyrosine phosphatases (HCPTP) A and B are 158 residue splicing variants belonging to the low molecular weight family of protein tyrosine phosphatases. HCPTP-A and HCPTP-B are 87.3% identical, containing a 34 residue variable region, and are structurally very similar with an overall RMSD = 0.63 Å. HCPTP is found to be overexpressed in a number of cancers, specifically those which also overexpress its primary target protein, EphA2. Overexpression of inactive HCPTP D129A in transformed MDA-MB-231 cells is correlated with a reduction in EphA2 expression and results in the reversion of these cells back to a non-transformed phenotype. The link between HCPTP and metastatic transformation makes HCPTP a suitable drug target. However, despite structurally similar active sites and a conserved CX₅R signature sequence found in all phosphatases, HCPTP-A and HCPTP-B have drastically different activities towards p-nitrophenyl-phosphate and have been referred to as fast and slow isoenzymes, respectively. The recent discovery of a secondary binding site found 12 Å away from the active site has given rise to the possibility that bifunctional inhibitors could be designed that could discriminate between these splice variants. In addition to differences in activity, the HCPTP variants have been proposed to differ in EphA2 tyrosine specificity and subsequently to have differing effects on downstream signaling. We used a mass spectrometry based assay to determine relative rates of dephosphorylation for the two HCPTP variants at phosphotyrosine sites associated with control of EphA2 activity and downstream signaling. Our results show that tyrosine Y960 in the SAM domain is dephosphorylated exclusively by HCPTP-B while the activation loop tyrosine Y772 is dephosphorylated 6 times faster by HCPTP-A. In contrast, the juxtamembrane tyrosines Y575, Y588, and Y594 are dephosphorylated by both variants with similar rates. The difference in EphA2 dephosphorylation indicates different roles for the two HCPTP variants and presents a need to characterize each within the cell. To address this, we have designed variant specific peptide antibodies against HCPTP that will be used to determine the location and expression of each HCPTP variant in non-transformed and transformed cell lines. Understanding which HCPTP variant plays a role in cell transformation will be critical for the development of effective drug candidates against HCPTP.

1, 25 Dihydroxyvitamin D Regulation of Energy Metabolism in MCF10 Human Breast Epithelial Cells

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These studies were designed to investigate the impact of 1,25 dihydroxyvitamin D (1,25D) on a critical shift to cancer progression mediated by hypoxia inducible factor-1 α (HIF-1 α). HIF-1 α reduces oxidative metabolism by inducing pyruvate dehydrogenase kinase 1 (PDK1), promoting lactate production by activating lactate dehydrogenase (LDH), and increasing glycolysis. A cell model for breast cancer progression including untransformed (Unt), *ras*-oncogene transfected (*ras*) and malignant (CA1h) MCF10 human breast epithelial cells was used. 1,25D reduced HIF-1 α protein by 17% and 36%, reduced LDH activity by 11% and 8% in *ras* and CA1h, respectively, as well as a trend towards decreased PDK1 protein level in both cell lines. A highly sensitive non-invasive micro biosensor in self-referencing mode was employed to measure physiological glucose, oxygen and lactate flux at the cell membrane. Results showed glucose flux was approximately 2-fold higher in *ras* cells than Unt cells, characteristic of the Warburg effect. Four day 1,25D treatment reduced oxygen uptake by 20% in the basal condition, and induced glucose (25%) and oxygen uptake (22%) in response to additional glucose (10 μ M) compared to vehicle in *ras* cells. However, with increasing doses and uptake of glucose, lactate efflux was similar between vehicle and 1,25D treated *ras* cells. These studies suggest 1,25D may shift glucose utilization towards increased glucose and oxygen uptake, but not lactate production, thus may regulate energy metabolism in cancer progression.

USE OF GENOMIC DATA TO DRIVE RATIONALE FOR TARGETING PCNA-FEN1 INTERACTIONS

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Implementation of genetic staging of breast cancer patients has aided in bring the era of personalized medicine into reality. Advancement of personalized medicine for breast cancer patients has been challenged by the emergence of drug resistance. The use of genomic data is hypothesized to help provide rational pathways for development of combination therapies that overall reduce the emergence of drug resistance for a particular tumor genotype. A new strategy for designing a therapeutic strategy for triple-negative breast cancers is to design an inhibitor of a specific PCNA-protein interaction that is needed for efficient DNA repair. The use of The Cancer Genome Atlas and Kaplan Meier Plotter databases were used to identify specific PCNA interacting proteins (PIPs) that are overexpressed in triple-negative breast cancers and have been correlated to poor prognosis. Out of the 33 different tumors analyzed in The Cancer Genome Atlas, *FEN1* was demonstrated to be overexpressed in 70% of all triple-negative breast tumors. Furthermore, this overexpression yielded a worse prognosis than those patients bearing tumors with low expression of *FEN1* ($p=0.0528$, $n=609$). Together these results suggest that in triple-negative breast cancers, the FEN1-PCNA interaction may be very important in the proper repair caused by DNA damage.