5th Great Lakes Nuclear Receptor Conference (GLNRC)

Friday & Saturday, October 12-13, 2012

Northwestern University
Prentice Women’s Hospital
Robert H. Lurie Medical Research Center
Chicago, IL

Program and Abstract Book

Organizers:
Debabrata (Debu) Chakravarti, Northwestern University
Jonna Frasor, University of Illinois at Chicago
5th Great Lakes Nuclear Receptor Conference

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Dear Colleagues:

A very warm welcome to the beautiful city of Chicago and the Fifth Biennial Great Lakes Nuclear Receptor Conference (GLNRC-2012) to be held on October 12-13, 2012 at Northwestern University. The theme of the meeting is “Nuclear Receptors and Hormone Signaling: Mechanisms, Physiology, and Human Diseases”. We are especially excited that we have partnered with another prestigious regional meeting, the Illinois Symposium on Reproductive Sciences (ISRS), to maximize the impact of this meeting for each of our respective fields. Please note that GLNRC and ISRS will share the keynote address and reception on Friday (Oct 12) afternoon and evening. Dr. Ken Korach of NIEHS will deliver the joint keynote lecture on Oct 12, 2012 and Dr. Geoffrey Greene of University of Chicago will deliver the GLNRC keynote lecture on October 13, 2012. This joint conference offers our trainees a rich opportunity to present their work to a diverse but complimentary and multidisciplinary audience of junior and senior scientists from across the upper Midwest in a highly interactive setting.

In addition to the two outstanding keynote lectures, we have put together a very strong program with oral presentations mostly by junior faculty members, graduate students, and postdoctoral fellows, as well as two poster sessions that allow ample time for interactions. GLNRC-2012 will host a record number of participants: a total of 140 graduate students, postdoctoral fellows and faculty members for this event. We received over 60 abstracts and all 15 oral presentations were selected from the submitted abstracts. The quality of submitted abstracts is very high, which will become evident when you visit individual posters and listen to the selected talks during the meeting.

We would like to express our gratitude to the Endocrine Society for endorsing our meeting and for their generous support of 10 travel awards and 5 complimentary Endocrine Society trainee memberships. We are also thankful for the generous support from the cancer centers of Northwestern University and University of Illinois and the office of the Associate Dean for Research and Graduate Studies of Weinberg College of Arts and Sciences of Northwestern University. We also thank the following Departments and Centers for their financial help: Department of Physiology, UIC, Department of OB/GYN, and Center for Genetic Medicine, Division of Endocrinology, and Signal Transduction in Cancer Program of the Cancer Center, Northwestern University. We are proud to inform you that GLNRC2012 is highlighted by the Journal, Molecular Endocrinology, under its News and Updates. Finally, we thank our corporate sponsors, IDT and Life Technologies for their support and encourage you to visit their exhibit tables.

This is your conference and its continued success hinges on your active involvement and participation. We hope you will have a wonderful time at the meeting and visiting Chicago.

Thank you very much for your participation and we look forward to seeing you at the next meeting in 2014.

Sincerely yours,

Debu Chakravarti, Ph.D.
Vice Director, and Associate Professor
Division of Reproductive Biology Research
Leader, Hormone Action and Signal Transduction Program
Robert H. Lurie Comprehensive Cancer Center
Northwestern University Feinberg School of Medicine
Chicago, IL 60611

Jonna Frasor, Ph.D
Associate Professor
Department of Physiology and Biophysics
University of Illinois at Chicago

Co-organizers of GLNRC2012
FRIDAY, OCTOBER 12

Friday Sessions will be in the Lurie Medical Research Center, Northwestern University at 303 E. Superior St., Chicago. Keynote address will be in the Hughes Auditorium on the ground floor. Reception will be in the Ryan Family Atrium outside the auditorium.

3:00 pm  GLNRC registration table opens. Ryan Family Atrium on ground floor of Lurie Medical Research Center.

4:15 pm  GLNRC/ISRS Joint Session
Opening Remarks from Great Lakes Nuclear Receptor Conference organizers. Lurie Research Medical Research Center - Hughes Auditorium.

4:30 pm  GLNRC/ISRS Joint Keynote Lecture
"Analyzing the Functional Domains of the Estrogen Receptor in Estrogen Biology"
Neena B. Schwartz Lectureship in Reproductive Science given by Kenneth Korach, Ph.D., Chief Laboratory of Reproductive and Developmental Toxicology, NIEHS/NIH

5:30 pm  ISRS announcements, award presentations, and closing remarks

5:45 pm  GLNRC/ISRS Joint Reception
Ryan Family Atrium, Lurie Medical Research Building
hot & cold hors d’oeuvres, wine, beer, and soft drinks
ISRS & some GLNRC posters available for viewing

Sign-up for “Dinner on the Town”
Group reservations at a set of diverse & great Chicago restaurants nearby have already been made. This is an opportunity to meet new colleagues. Sign-up for the restaurant of your choice and have dinner with a mix of junior and senior scientists.

7:30 pm  GLNRC/ISRS Joint “Dinner on the Town”.
Cost of dinner is not included in registration. If you mounted your poster for the reception, it must be taken down by this time and then brought to the Prentice Hospital site on Saturday morning.
SATURDAY, OCTOBER 13

Saturday sessions will be held on the 3rd floor of Prentice Women's Hospital at 250 E. Superior St. Oral sessions will be in the Canning Auditorium. Posters and lunch will be in the Levin Auditorium.

7:30 am  Continental Breakfast and Poster set-up, Levin Auditorium
           Registration, at entrance to Levin Auditorium, Prentice Women's Hospital

8:30 am  Opening Remarks
           Canning Auditorium, Prentice Women's Hospital

GLNRC Oral Session 1: Nuclear Receptors in Physiology and Disease
           Canning Auditorium, Prentice Women's Hospital
           Session Chairs: Young Jeong, University of Illinois at Chicago and Julie Kim, Northwestern University

8:40 am  Repurposing of Anti-Inflammatory Selective Glucocorticoid Receptor Activator Compound A as Anti-Lymphoma Drug
           Gleb Baida, Northwestern University, Chicago, IL

8:55 am  Steroid Sulfatase Enhances Hepatic Estrogen Activity and Protects Mice from Obesity and Type 2 Diabetes
           Mengxi Jiang, University of Pittsburgh, Pittsburgh, PA.

9:10 am  Intestinal Epithelial VDR is Sufficient to Inhibit Experimental Colitis Independent of Immune VDR Actions
           Weicheng Liu, The University of Chicago, Chicago, IL

9:25 am  The NR4A Orphan Nuclear Receptors Repress the Inhibin A Subunit Gene Through the Transcription Factor GATA-4 in Ovarian Granulosa Cells.
           Kristin M. Meldi, Northwestern University, Evanston, IL.

9:40 am  The Nuclear Receptor Cochaperone FKBP51 is Required for Diet-Induced Visceral Adiposity
           Lance A. Stechschulte, University of Toledo College of Medicine, Toledo, OH

9:55 am  Coffee Break
           Levin Auditorium, Prentice Women's Hospital

GLNRC Oral Session II: Nuclear Receptors and Cancer
           Canning Auditorium, Prentice Women's Hospital
           Session Chairs: Stephen Hammes, University of Rochester and Wei Xu, University of Wisconsin
10:15 am  Paxillin is a Universal and Essential Regulator of Androgen-Mediated Processes  
Stephen R Hammes, University of Rochester School of Medicine, Rochester, NY

10:30 am  Hsp90 Inhibition Targets Glucocorticoid Receptor (GR) Activity And Reverses Chemotherapy Resistance In GR+ Triple Negative Breast Cancer  
Abena S. Agyeman, University of Chicago, Chicago, IL.

10:45 am  Genome Wide RNAi Screen to Understand Estrogen Mediated Proliferation in Breast Cancer  
Ruby Dhar, University of Chicago, Chicago, IL

11:00 am  EZH2 activation of AR/AR transcriptional signaling in metastatic prostate cancer  
Jung Kim, Northwestern University, Chicago, IL

11:15 am  Steroidogenic Factor 1 Expression Induces Steroid Synthesis to Fuel Growth of Prostate Cancer Cells  
Samantha R. Lewis, University of Wisconsin, Madison, WI

11:30 am  Poster Session A (odd # posters)  
Levin Auditorium, Prentice Women’s Hospital

12:15 pm  Lunch  
Levin Auditorium, Prentice Women’s Hospital

1:15 pm  Poster Session B (even # posters)  
Levin Auditorium, Prentice Women’s Hospital

GLNRC Oral Session III: Nuclear Receptors: Mechanisms of Action  
Canning Auditorium, Prentice Women's Hospital  
Session Chairs: Grant Barish, Northwestern University and Eric Bolton, UIUC

2:00 pm  Establish a Cellular TR-FRET Assay to Screen Allosteric Activators of CARM1 for Breast Cancer Differentiation Therapy  
Hao Zeng, University of Wisconsin, Madison, WI

2:15 pm  Regulation of Cell Proliferation by Androgen Receptor Signaling  
Eric C. Bolton, University of Illinois at Urbana-Champaign, Urbana, IL

2:30 pm  Biochemical Analysis of Androgen Receptor Transcriptional Complexes using Quantitative Mass Spectrometry  
Jordy J. Hsiao, University of Iowa Carver College of Medicine, Iowa City, IA
2:45 pm  **Prolyl Isomerase Pin1 Inhibits Phosphorylation-dependent Ubiquitination and Degradation of ERα**
Prashant Rajbhandari, University of Wisconsin-Madison, Madison, WI

3:00 pm  **A Novel Histone Deacetylase Complex Plays both Coactivator and Corepressor Roles in AIB1 Transcription**
Jinsong Zhang, University of Cincinnati, Cincinnati, OH

3:30 pm  **GLNRC Keynote Lecture**
Canning Auditorium, Prentice Women's Hospital
Geoffrey Greene, Professor and Vice Chair, The Ben May Department of Cancer Research, University of Chicago
"Targeting Nuclear Receptors in Health and Disease: When Discrimination is Good"

4:30 pm  **Closing Remarks**

4:45 pm  **Closing Reception**
Harris Family Atrium, 3rd floor, Prentice Women's Hospital
Hot and cold hors d'oeuvres and soft drinks

6:00 pm  **Meeting ends. Please take down all posters by this time.**
Kenneth S. Korach currently is the Director of the Environmental Disease and Medicine Program, and Chief of the Laboratory of Reproductive and Developmental Toxicology at the National Institute of Environmental Health Sciences (NIEHS). He received his Ph.D. in Endocrinology from the Medical College of Georgia where he characterized biochemical properties of estrogen receptors in the pituitary and hypothalamus. Dr. Korach was a Biological Chemistry Postdoctoral and a Ford Research Fellow at Harvard Medical School with Professor Lewis Engel. Ken joined the NIEHS where he has headed a research group investigating estrogen hormone action in numerous tissues by the generation of the ERKO mouse models with an application towards understanding the basic mechanisms of estrogen’s influence on physiological processes. His contribution in the field of estrogen action and Nuclear Receptor biology is outstanding. Dr. Korach was an Editor and past Editor-in-Chief for Endocrinology. He holds multiple adjunct Professorships at North Carolina State University, the University of North Carolina Medical School and in Pharmacology and Cancer Biology at Duke University Medical School. He was appointed into the Senior Biomedical Research Service (SBRS) at the NIH. He is the recipient of the National Institutes of Health outstanding performance awards, National Institutes of Health Merit Awards, the Medical College of Georgia Distinguished Alumnus Award, the Edwin B. Astwood Award from the Endocrine Society, the Keith Harrison Lecture Award from the Australian Endocrine Society, the Transatlantic Medal from the British Endocrine Society, Firkin Research Award from the Australian Society of Medical Research, Dale Research Medal from the Society of Endocrinology, and the Beacon Award from the Frontiers in Reproduction Woods Hole Research Laboratories.

Lecture title:

Analyzing the Functional Domains of the ER in Estrogen Biology
Dr. Greene received his B.A. in chemistry from the College of Wooster and his Ph.D. in organic chemistry from Northwestern University. He joined the Ben May Laboratories faculty as an Assistant Professor in 1980 after receiving postdoctoral training in Dr. Elwood Jensen’s laboratory at the University of Chicago. He was promoted to full Professor in the Ben May Department for Cancer Research in 1991 and is currently Vice Chair. He is also Chair of the Committee on Cancer Biology and Co-Director of the Ludwig Center for Metastasis Research. His lab studies the molecular mechanisms by which steroids and SERMs regulate development, differentiation, cellular proliferation and survival in hormone responsive tissues and cancers, via one or both of the two estrogen receptor subtypes, ERα & ERβ.

Lecture Title:

Targeting Nuclear Receptors in Health and Disease: When Discrimination is Good

Abstract: Natural and synthetic ligands regulate diverse signaling and transcriptional networks via many members of the nuclear receptor (NR) superfamily, including the two estrogen receptor subtypes, ERα and ERβ. The structural and functional events underlying ligand-specific co-regulator recruitment and resultant transcriptional activation or repression are complex and still not well defined for the NRs. Subtype and ligand-specific recruitment of co-regulators and modulation of target gene expression have important implications for understanding the specificity of nuclear receptor signalling, for the treatment or prevention of a variety of diseases, and for endocrine disruption. The development of diverse ER subtype-selective ligands has provided both powerful molecular tools as well as drugs with clinical utility. The same drugs, like tamoxifen or raloxifene, can be used to treat hormone-sensitive breast cancers, as chemoprevention agents to reduce the risk of breast cancer and as drugs to maintain bone density and prevent fractures. Other SERMs have potential as anti-inflammatory agents. Structural information for ERα and ERβ ligand-binding domains (LBDs) occupied by these ligands has helped define the molecular and structural differences that account for these diverse applications. Detailed structure-function information for the two ERs has proved useful both for understanding as well as designing ligands with tissue- and pathway-selective behaviors. Combined with knowledge gained from extensive genomic and proteomic mapping studies of target genes and interactors for both ER subtypes, it should be possible to identify additional applications for existing drugs. In addition, certain combinations of estrogens and SERMs may be able to provide multiple health benefits simultaneously.
Abstracts:

Oral Presentations
Activation of nutritional sensor REDD1 is necessary for the development of steroid-induced cutaneous atrophy.

G. Baida1, R. Lavker1, P. Bhalla1, T. Finkel3, E. Lesovaya4, K. Yuen1, I. Budunova1

1 Department of Dermatology, Northwestern University, Chicago, IL, USA
2 N. Blokhin Cancer Research Center, RAMS, Moscow, Russia
3 CHOP, Philadelphia, Pennsylvania, USA

One of the major adverse effects of topical glucocorticoids is cutaneous atrophy. The molecular mechanisms underlying this phenomenon are poorly understood. To elucidate the mechanisms of epidermal hypoplasia, we used chronic topical treatment of mouse and human skin with glucocorticoids which resulted in marked epidermal thinning and depletion of the interfollicular keratinocyte population. We found that a single exposure or chronic treatment with glucocorticoids induced a strong increase in the expression of REDD1 (regulated in development and DNA damage response) at mRNA and protein levels in mouse and human epidermis. REDD1 is a nutritional sensor and a stress-inducible inhibitor of mTOR complex 1, critical for protein synthesis regulation. Accordingly, we also noted a strong decrease in phospho-mTORS2448 expression in epidermis after exposure to glucocorticoids. REDD1 is a primary target gene for the glucocorticoid receptor (GR), whose expression is regulated via GR binding sites in the REDD1 promoter. We observed that glucocorticoids strongly induced expression of TXNIP (thioredoxin-interacting protein) that binds to and stabilizes REDD1 protein. We also observed down regulation of conservative miRNA-221 that regulates REDD1 expression. Most importantly, REDD1 knockout animals appeared resistant to epidermal atrophy induced by glucocorticoids. Finally, we revealed an excellent correlation between REDD1 induction in skin and individual animal sensitivity to adverse effects of topical steroids. Collectively, our findings reveal a novel mechanism of glucocorticoid-induced skin atrophy and link glucocorticoids to the control of protein synthesis in keratinocytes via mTOR regulation. In addition, our findings are relevant to the development of selective GR activators that have safer therapeutic profiles than classical steroids. Supported by Supported by RO1CA118890 (to I.B. and R.L.).
Steroid sulfatase enhances hepatic estrogen activity and protects mice from obesity and type 2 diabetes

Mengxi Jiang¹ and Wen Xie¹, ²,*

¹ Center for Pharmacogenetics and Department of Pharmaceutical Sciences
² Department of Pharmacology and Chemical Biology, University of Pittsburgh, Pittsburgh, PA 15261, USA.

The enzyme steroid sulfatase (STS) is responsible for the formation of biologically active estrogens by the hydrolysis of estrogen sulfates and regulates estrogen homeostasis. In this report, we showed that hepatic over-expression of STS in female STS transgenic mice elicited metabolic benefits that protected mice from high fat diet (HFD) induced obesity and type 2 diabetes. STS transgenic mice subjected to HFD challenge displayed significant decrease in fat mass and body weight, and increased energy expenditure without alterations in food intake compared with their wild-type counterparts. Moreover, STS transgenic mice had improved glucose tolerance. The metabolic benefit was associated with inhibition of hepatic gluconeogenic genes and inflammation related genes. Silencing the transgene expression through tet-off regulatory system abolished the metabolic benefit, indicating that the protection is caused by transgene itself rather than non-specific effects from random transgene insertion sites. The hepatic estrogen signaling was enhanced in STS transgenic mice, whereas elimination of the primary source of estrogens by ovariectomy abolished the protective metabolic phenotype, suggesting the metabolic action is mediated by increased estrogenic activity in STS transgenic mice liver. In summary, our results have revealed an important metabolic function of STS and may establish it as a novel therapeutic target for the prevention and treatment of obesity and type 2 diabetes. Supported by NIH grants ES014626, ES019629 and DK083952.
Intestinal epithelial VDR is sufficient to inhibit experimental colitis independent of immune VDR actions

Weicheng Liu, Yunzi Chen, Maria L. Annunziata, Ursula Dougherty, Maya Aharoni Golan, Mark Musch, Juan Kong, Marc Bissonnette, Stephen B. Hanauer and Yan Chun Li

Section of Gastroenterology, Hepatology and Nutrition, Department of Medicine, Division of Biological Sciences, The University of Chicago, Chicago, IL 60637, USA

Background: Epidemiological evidence demonstrates vitamin D-deficiency is highly prevalent in IBD and vitamin D status is inversely associated with disease severity, the mechanistic impact(s) of vitamin D deficiency remains unclear. Vitamin D hormone activity is mediated by the vitamin D receptor (VDR). Previous studies using IL-10(-/-)/VDR(-/-) mice concluded that VDR signaling in immune cells inhibits colonic inflammation and colitis development. In addition, our investigations of VDR(-/-) mice suggested that the epithelial VDR attenuates experimental colitis by protecting the mucosal epithelial barrier and that colonic epithelial VDR levels were markedly reduced in the biopsies from patients with IBD.

Aims: To identify the anti-colitic role of the colonic epithelial vs. immune VDR signaling in the development of experimental colitis.

Methods: Transgenic (Tg) mice were generated in which villin promoter was used to target Flag-tagged human (h)VDR to intestinal epithelial cells (IEC). Transgenic-VDR(-/-) (Tg-KO) mice were also produced. Tg, Tg-KO, VDR(-/-) and wild-type (WT) mice were studied in parallel using TNBS- and DSS-colitis models.

Results: In both TNBS and DSS models, Tg mice were highly resistant to colitis induction compared to WT mice, manifested by lower clinical and histological scores and marked reduction in colonic epithelial damage and inflammatory cytokine production. Colonic epithelial permeability was well preserved in Tg mice. VDR(-/-) mice developed the most severe colitis that led to high mortality in both models, whereas Tg-KO mice were highly resistant to colitis with almost no mortality. Mechanistically, VDR overexpression attenuated TNBS- or DSS-induced IEC apoptosis, with marked suppression of PUMA induction and caspase 3 activation. In vitro and in vivo data demonstrated that VDR signaling inhibited PUMA expression by blocking NF-kB activation.

Conclusions: These results demonstrate that intestinal epithelial VDR signaling inhibits colonic inflammation by protecting the integrity of the mucosal epithelial barrier. The observation that the hVDR transgene rescues VDR-null mice from developing severe colitis demonstrates that the anti-colitic activity of the epithelial VDR is independent of the immune VDR actions.
The NR4A orphan nuclear receptors repress the inhibin α subunit gene through the transcription factor GATA-4 in ovarian granulosa cells.

K.M. Meldi, A.D. Burkart-Sadusky, W.B. Pearse, and K.E. Mayo.

Department of Molecular Biosciences, Center for Reproductive Science, Northwestern University, Evanston, IL.

In the ovary, nuclear receptors act as important transcriptional regulators of many genes in response to follicle-stimulating hormone (FSH) and luteinizing hormone (LH). The NR5A orphan nuclear receptors liver receptor homolog 1 (LRH-1) and steroidogenic factor 1 (SF-1) activate expression of the inhibin α subunit gene in response to FSH. Upon the LH surge, inhibin α gene expression must be repressed to permit the secondary FSH surge. The NR4A nuclear receptors nerve growth factor inducible B (NGFI-B), nuclear receptor related 1 (NURR1), and neuron-derived orphan receptor 1 (NOR-1) are rapidly induced at a time when inhibin α mRNA is reduced. We explored the role of these receptors in regulation of the inhibin α gene. We show that the NR4A receptors repress inhibin α promoter activity in a mouse granulosa cell line, GRMO2. Electrophoretic gel mobility shift assays show the NR4A receptors do not bind to the inhibin α promoter in vitro. Mutations within the DNA-binding domains of the receptors do not abolish NR4A-mediated repression, confirming that it is mediated independently of DNA binding. We show that the upstream (5’) GATA binding site within the proximal inhibin α promoter that binds the transcription factor GATA-4 is critical for NR4A-mediated repression of the gene. Co-immunoprecipitation assays demonstrate the NR4A receptors physically interact with GATA-4. This interaction occurs on the DNA, as NGFI-B is recruited to the inhibin α GATA site by GATA-4 in DNA pulldown assays. Therefore, the NR4A receptors are physically recruited to the inhibin α promoter through GATA-4. Ongoing experiments are focused on characterizing the interaction domains between the NR4A receptors and GATA-4 to determine how this interaction results in gene repression. This research was supported by the Eunice Kennedy Shriver NICHD/NIH through cooperative agreement U54 HD041857 as part of the Specialized Cooperative Centers Program in Reproduction and Infertility Research to KEM.
The nuclear receptor cochaperone FKBP51 is required for diet-induced visceral adiposity

Lance A. Stechschulte, Manya Warrier, Terry D. Hinds Jr., and Edwin R. Sanchez

Department of Physiology & Pharmacology, and Center for Diabetes & Endocrine Research, University of Toledo College of Medicine, Toledo, OH 43164

Nuclear receptors (NRs) are hormone-regulated transcription factors that translate endocrine, metabolic, nutritional, developmental, and pathophysiological signals into gene regulation. NRs form heterocomplexes with Hsp90 and several cochaperones, including FK506-binding protein-51 (FKBP51), FKBP52, PP5, and Cyp40. We have evidence that loss of FKBP51 increases glucocorticoid receptor (GR) and decreases peroxisome proliferator-activated receptor-γ (PPARγ) transcriptional activity. In this study, we investigate the hypothesis that loss of FKBP51 leads to reciprocal dysregulation of GR and PPARγ, thereby increasing the expression of genes regulating lipid oxidation and/or energy expenditure. FKBP51-KO mice were subjected to a high fat diet, compared to wild-type mice, FKBP51-KO animals were resistant to diet-induced obesity, with reduced visceral adiposity, serum triglyceride, and free fatty acids. Food consumption, insulin secretion and clearance, and hepatic lipid accumulation were unchanged. Gene profiling of adipose showed elevated expression of thermogenic genes in the KO mice. In vitro adipogenesis studies showed FKBP51-KO cells to be highly resistant to differentiation and lipid accumulation. The phosphorylation states of GR were tested using phospho-specific antibodies for serines 212, 220 and 234 – the major sites of hormone-induced phosphorylation. Results show FKBP51 controls GR phosphorylation at serines 220 and 234. The phosphorylation state of PPARγ were also tested using phosho-specific antibody for serine 112. Results show that knockdown of FKBP51 in differentiated 3T3-L1 cells resulted in hyperphosphorylation of serine 112. These investigations have uncovered FKBP51 as a key regulator of adipogenesis and thermogenesis and may serve as a new drug target in the treatment of obesity and related disorders. Supported by NIH grant DK70127 to E.R.S.
Paxillin is a universal and essential regulator of androgen-mediated processes

Stephen R Hammes and Aritro Sen

Division of Endocrinology and Metabolism, University of Rochester School of Medicine, Rochester, NY 14642

Steroid signaling occurs inside (genomic) and outside (nongenomic) the nucleus. While genomic signaling involves alterations in transcription, nongenomic signaling includes modulation of membrane-initiated pathways such as G protein and kinase signaling. We study androgen signaling, from its ability to promote oocyte meiosis to its role in ovarian follicle growth and its importance in prostate cancer proliferation. Surprisingly, we find that androgens use a conserved pathway for all of these processes that involves extranuclear activation of MAPK followed by changes in intranuclear transcription. Importantly, without extranuclear signaling, transcription and downstream physiologic events do not occur, suggesting a linear pathway whereby nongenomic androgen events are required for genomic androgen events. We now find that paxillin, a known cytoplasmic protein regulating cell adhesion, also serves as a “liaison” between extranuclear and intranuclear androgen signaling. Androgens promote transactivation of the EGF receptor, leading to Src activation. Src then phosphorylates paxillin on tyrosines, allowing MAPK to be activated. MAPK then promotes phosphorylation of paxillin on serines, after which paxillin translocates to the nucleus and regulates AR nuclear localization and AR-mediated transcription. In prostate cancer cells, if MAPK-dependent phosphorylation of paxillin cannot occur, then androgen-mediated transcription and proliferation are markedly reduced. In fact, this paxillin-regulated pathway is upregulated in human prostate cancer, demonstrating its potential importance in tumor biology. Interestingly, in the mouse ovary, MAPK and paxillin are required for androgen-induced granulosa cell proliferation and follicle growth, demonstrating the conserved nature of this pathway. In summary, our laboratory has discovered a novel conserved function of paxillin that will likely be important for a myriad of androgen-mediated processes. Supported by the NIH.
Hsp90 inhibition targets glucocorticoid receptor (GR) activity and reverses chemotherapy resistance in GR+ triple negative breast cancer.

A.S. Agyeman¹, N.D. Lee¹, D.A. Proia², S.D. Conzen¹

¹ Department of Medicine, The University of Chicago, Chicago, IL.
² Synta Pharmaceuticals Corp, Lexington, MA

A significant proportion of triple negative breast cancers (TNBCs) are chemotherapy-resistant. The molecular mechanisms underlying this resistance remain poorly understood. Our laboratory has previously demonstrated that a subset of TNBCs express the glucocorticoid receptor (GR) and that subsequent GR activation in these TNBCs upregulates several anti-apoptotic GR target genes. In addition, a meta-analysis of tumor gene expression and clinical outcome in women with early-stage TNBC found that high primary tumor GR-encoding gene (NR3C1) expression (GR+ TNBC) is associated with a significantly higher relapse rate compared to women with GR-negative TNBC. GR is a cortisol-activated nuclear receptor and an Hsp90 client protein that is rapidly targeted for degradation in cells treated with Hsp90 inhibitors. Therefore, we hypothesized that reducing GR activity in TNBCs using Hsp90 inhibitor treatment might increase tumor cell chemotherapy sensitivity. Although clinical use of first generation Hsp90 inhibitors is limited by toxicity, ganetespib (Synta) is a resorcinolic triazolone Hsp90 inhibitor that appears to have a significantly improved safety profile. Here we report that in vitro treatment of MDA-MB-231 TNBC cells with ganetespib and paclitaxel resulted in significant cytotoxic synergy. Furthermore, ganetespib treatment resulted in rapidly reduced total steady-state GR expression levels and persistent loss of GR Ser211 phosphorylation. By stably expressing a doxycycline-inducible shRNA to generate GR-depleted MDA-MB-231 cells, we also found that GR expression was required for ganetespib/paclitaxel cytotoxic synergy. Taken together, these data suggest that GR activity is an important target of Hsp90 inhibitor action in chemotherapy-resistant TNBC. Supported by NIH grant R21-CA149472.
Genome wide RNAi screen to understand estrogen mediated proliferation in Breast Cancer.

Ruby Dhar and Kevin P. White

IGSB, University of Chicago, Chicago, IL, USA, 60637

Breast cancer is rated as the second leading cause of cancer death (15%) among women worldwide and is thought to involve a complex interplay of genetic, hormonal and environmental factors. Substantial experimental, epidemiological and clinical evidences show that risk to breast cancer is influenced by estrogen (E2). However the molecular mechanisms underlying this are largely unknown. Present study is undertaken to delineate the alterations in genetic network involved in estrogen mediated enhanced proliferation in breast cancer.

We initiated a “systems level” whole genome functional analysis using RNA interference (RNAi) screen by assessing the proliferation of MCF-7 cells using BrdU incorporation both in absence and presence of estrogen. Results of our primary screen identified several novel candidate genes involved in E2 mediated proliferation which were further validated during our secondary and tertiary screens. To better understand the transcriptional and signaling components required for estrogen mediated proliferation, we did further analysis on a set of phosphatases and DNA binding factors. Knockdown of the former targets showed an increase while the later showed a decrease in estrogen mediated proliferation. Proteomic and genomic approaches were employed to find potential therapeutic targets for the set of estrogen specific suppressors and enhancers. Using western, we found the involvement of Akt, Erk2/4 as some of the target kinases for the phosphatases. In vivo binding of the transcription factors using ChIP –Seq showed GATA2 to be an important co-factor for ER-α and RXR family of Nuclear receptors. This study will eventually help in building a gene network to elucidate the entire ramification of E2 mediated signaling in breast cancer.

This work has been supported by KPW Beckman Startup grant, Breast Spore grant and P50 grant.
Androgen receptor (AR), a family member of nuclear hormone receptors, plays an important role in prostate cancer progression through activation of oncogenes, such as PSA and TMPRSS2. Enhancer of Zeste homolog 2 (EZH2) is a histone methyltransferase that is highly overexpressed in metastatic prostate cancer patients and methylate tumor suppressors at histone3 lysine27 (H3K27) to promote tumor progression. Previously, our lab have identified that EZH2 regulates AR in prostate cancer. However, the molecular mechanism of how EZH2 regulates AR was not identified. Therefore, in this study, we used LAPC4 as our model system to understand the molecular mechanism of how EZH2 regulates AR transcriptional activity. First, we identified miR-30e as an intermediate factor of EZH2 and AR using bioinformatics approach. Then, we used GSEA and molecular approaches to reveal that EZH2 regulation of AR is indeed through miR-30e. Here, our study shows that EZH2 inhibit miR-30e at the promoter region, therefore, inhibiting miR-30e to target AR at AR 3’UTR. This study provides an important link between EZH2 and AR, two proteins that plays an important role in prostate cancer progression.
Steroidogenic Factor 1 Expression Induces Steroid Synthesis to Fuel Growth of Prostate Cancer Cells

Samantha R. Lewis¹, William A. Ricke², Wei Huang³, and Joan S. Jorgensen¹

Departments of Comparative Biosciences¹, Urology², and Pathology and Laboratory Medicine³, University of Wisconsin, Madison, WI, USA

Although androgen deprivation therapy by medical or surgical castration is a cornerstone of treatment for metastatic prostate cancer, remission is only temporary and tumors inevitably progress to castration resistant prostate cancer (CRPC). There are several theories to explain this aggressive resurgence, one of which suggests that prostate adenocarcinoma cells acquire the machinery for de novo steroidogenesis. During development and throughout life, Steroidogenic Factor 1 (SF1, NR5A1) is a key regulator of steroidogenesis in normal endocrine tissues. Normal and benign prostate tissues lacks SF1 expression and are non-steroidogenic. Therefore, we hypothesized that SF1 was expressed in CRPC to stimulate steroidogenesis and fuel malignant growth. We found that SF1 was present in aggressive prostate cancer but absent in benign prostate epithelial cell lines. These results were supported by detection of SF1 in prostate biopsies from human patients with CRPC, whereas it was absent from localized prostate cancer and benign prostate hyperplasia. The functional role of SF1 was tested by overexpressing versus knocking down SF1 within benign (BPH-1) and CRPC (WR3) model cell lines respectively. Benign BPH-1 cells expressing ectopic SF1 exhibited increased mRNA levels of steroidogenic enzymes and secreted estradiol. In addition, the presence of SF1 induced cell growth and proliferation. In contrast, aggressive WR3 prostate cancer cells harboring shRNA designed to target SF1 for knockdown (shSF1) expressed decreased transcripts of steroidogenic enzymes and exhibited slowed growth and proliferation. Based on these data, we conclude that SF1 is present in aggressive prostate cancers where it stimulates steroidogenesis to promote aggressive growth. Our findings present a new potential mechanism and therapeutic target for deadly prostate cancer.

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Establish a cellular TR-FRET assay to screen allosteric activators of CARM1 for breast cancer differentiation therapy

Hao Zeng, Kun Bi, Michael Hoffmann, Wei Xu

1McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue, Madison, WI 53706
2Life Technologies Corporation, Discovery and ADMET Systems, Madison, WI 53719, USA.

Coactivator Associated Arginine Methyltransferase 1 (CARM1) is involved in the activation of a number of cancer-relevant transcription factors including NF-kB, p53, E2F1, and steroid receptors, among which transcriptional activation of ERα by CARM1 is best characterized. We showed that overexpression of CARM1 by two-fold in MCF7 breast cancer cells increased the expression of ERα target genes involved in differentiation, reduced cell proliferation and reduced growth in soft agar. These results led to our paradigm-shifting hypothesis that activating CARM1 may be therapeutically effective in breast cancer. Selective, potent, cell-permeable CARM1 activators will be essential to test this hypothesis and to elucidate the roles of CARM1 in vitro and in vivo in cancer and epigenetic regulation.

Although several assays have been applied for the discovery of small-molecule inhibitors of CARM1, most of them relied on purified CARM1 and protein or peptide substrates, which could not recapitulate CARM1 cellular activity. In order to screen chemical activators of CARM1 for potential breast cancer therapy, we took advantage of LanthaScreen™, a high-throughput compatible time-resolved Förster resonance energy transfer (TR-FRET) assay developed by Life Technologies Inc. We developed a methyl-GFP-PABP1 based TR-FRET assay in which GFP fused PABP1 (GFP-PABP1) and a small amount of CARM1 are expressed in endogenous CARM1-depleted MCF7 cells via BacMam virus gene delivery system. The TR-FRET value, determined as a ratio of emission at 520nm over emission at 495nm, reflects the level of PABP1 methylation catalyzed by CARM1. We demonstrated that methyl-GFP-PABP1 TR-FRET signal is reflective of CARM1 expression and/or activity and Z’ factor was determined to be 0.76, which is suitable for high-throughput screening (HTS). We will soon implement the screen on ~50,000 compounds available at the Small Molecule Synthesis and Screening Facility (SMSSF) of UW-Madison. Small molecules identified from our screening may be developed as “epigenetic” drugs for ERα-positive breast cancers that failed conventional endocrine therapy.
Regulation of cell proliferation by androgen receptor signaling

Eric C. Bolton, Young-Chae Kim, Congcong Chen

Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL.

The androgen receptor (AR) is a ligand-activated transcriptional regulator that mediates the developmental, physiologic and pathologic effects of androgens. For example, dihydrotestosterone levels and AR expression are often elevated in prostate tumors to maintain the expression of andromedins such as growth factors and survival factors that influence cell cycle progression. However, the mechanisms of AR-mediated transcriptional regulation of those andromedins and other cell cycle regulators in prostate epithelial cells are not understood, though they are necessary for prostate development, function and disease progression.

Previously, we identified androgen responsive genes (ARGs) in HPr-1AR human prostate epithelial cells. Our objective here was to identify ARGs in prostate epithelial cells that control cell proliferation, and to investigate the mechanisms through which AR regulates their transcription. We examined the effects of androgens on cell proliferation and found that DHT inhibited HPr-1AR cell proliferation, increasing the doubling time more than 2-fold. Cyclin D gene products, which are crucial for cell cycle progression, were androgen-repressed. Indeed, Cyclin D mRNAs decreased with androgen treatment, and these effects were AR-dependent. Remarkably, Cyclin D1 mRNA was destabilized and exhibited a shorter half-life following androgen treatment, whereas Cyclin D2 mRNA stability was unchanged by androgen, suggesting a mechanism involving transcriptional repression. As Cyclin Ds have been implicated in the regulation of cell proliferation, our results suggest that in HPr-1AR cells the AR-mediated inhibition of cell proliferation by androgens may involve transcriptional repression and/or mRNA destabilization of Cyclin Ds. Our findings provide insight into the mechanisms of gene- and cell-specific transcriptional regulation operating within hormone-responsive gene networks, which modulate the physiologic and pathologic effects of hormones.
Biochemical analysis of androgen receptor transcriptional complexes using quantitative mass spectrometry

Jordy J. Hsiao, Harryl D. Martinez, Michael E. Wright

Department of Molecular Physiology and Biophysics, University of Iowa Carver College of Medicine, Iowa City, IA.

Aberrant androgen receptor (AR) activity is the primary driver of early- and late-stage human prostate cancers. Coregulators of AR-mediated transcription are critical determinants of aberrant AR activity in human prostate cancers. We have developed an analytical workflow to study the dynamic changes in coregulators involved in AR-mediated transcription to elucidate the molecular mechanism(s) of action of antiandrogens (i.e. selective androgen receptor modulator antagonists- SARM antagonists) used during androgen deprivation therapy (ADT). Nuclear protein extracts prepared from prostate cancer cells under androgen-depleted or androgen-stimulated conditions were used to isolate AR transcriptional complexes that copurified to the proximal promoter of the androgen-regulated rat probasin gene. Affinity-purified samples were subjected to sucrose density-gradient centrifugation, and DNA-bound protein complexes isolated were analyzed using label-free, directed mass spectrometry (dMS). Our proteomic screen validated known coregulators of AR-mediated transcription. Many coregulators were observed to be androgen-sensitive, which included chromatin remodeling proteins, enzymes involved in post-translational modification, Mediator complexes, and components of the RNA polymerase II transcriptional complex. The screen identified new coregulators of AR-mediated transcription that function in cellular signaling pathways unrelated to AR signaling in prostate cancer cells. We will utilize this biochemical workflow to understand the effects of SARM antagonists on coregulators involved in AR-mediated transcription. More importantly, we expect these studies will provide deeper mechanistic understanding of how coregulators impact tumor cell resistance to SARM antagonists in castration-resistant prostate cancers (CRPCs) that occurs in the ADT treatment of late-stage, metastatic prostate cancers. Supported by non-Federal Research Funds.
Prolyl isomerase Pin1 inhibits phosphorylation-dependent ubiquitination and degradation of ERα

Prashant Rajbhandari, Natalia M. Solodin, and Elaine T. Alarid

Department of Oncology and UW Carbone Comprehensive Cancer Center, University of Wisconsin-Madison, Madison, WI

Estrogen receptor-alpha (ERα) is an important protein biomarker that is used to classify breast cancers and predict patient response to hormonal therapies. Cellular levels of ERα protein modulate receptor activity and are controlled post-translationally by the ubiquitin-proteasome pathway (UPP). We previously showed that S118 in ERα is an essential cis-element regulating inducible ERα degradation by UPP. Phosphorylation at S118 is also necessary for full ERα transcriptional function but mutations at this site reveal differential regulation of ERα protein stability and transcription. How phosphorylation contributes to ERα regulation by the UPP has not been resolved. Phospho-S118 (pS118) is flanked by a proline residue, and binds the phosphorylation-dependent peptidyl prolyl isomerase, Pin1. Here, we demonstrate that Pin1 regulates steady state levels of ERα protein by disrupting ubiquitination and inhibiting proteasome-dependent receptor degradation. Like Pin1, the E3 ligase, E6AP, binds to ERα in an S118-dependent manner. Pin1 disrupts E6AP binding and prevents E6AP-mediated ubiquitination. Inhibition of Pin1 isomerase activity does not abrogate Pin1 disruption of E6AP-mediated ubiquitination, indicating that Pin1 binding but not isomerase activity is required to stabilize ERα protein. Pin1 isomerization activity is however necessary for Pin1-mediated regulation of ERα transactivation. Phospho-S118 and Pin1 play dual roles in the regulation of ERα protein and function and these data imply that Pin1 can function downstream of phosphorylation to independently regulate ERα fate and function.

This work is supported by NIH grants CA159578 (to E.T.A.) and T32 CA009135 (to P.R)
A novel histone deacetylase complex plays both coactivator and corepressor roles in AIB1 transcription

X. Duan*, C. Guo*, A. Gardner* (*: equal contribution), S. Khan1, J. Zhang1

1Department of Cancer and Cell Biology, University of Cincinnati, Cincinnati, OH.

Transcriptional overexpression of AIB1 (amplified in breast cancer 1), a breast cancer oncogene, drives cancer progression and anti-estrogen resistance. Here, we report the isolation and characterization of a novel histone deacetylase 1 (HDAC1) complex that serves as both coactivator and corepressor of AIB1 transcription. Unlike the previously-reported HDAC1 complexes, this complex has both histone deacetylase and acetyltransferase activities. Through the various components, this complex not only provides the essential driving force for AIB1 activation, but also negatively restricts the level of this activation. In addition to HDAC1 and HDAC2, the core complex also includes: i) p37, an HMG-like architectural DNA-binding protein, ii) BCAR2 (breast cancer anti-estrogen resistance protein 2), a previously characterized protein whose increased expression converts tamoxifen-sensitive breast cancer cells into resistant cells, and iii) SNAP1, an uncharacterized BCAR2-like protein. Both BCAR2 and SNAP1 contain conserved SANT and ELM2 domains to bind p37 and HDACs. Additionally, specific regions of BCAR2 link this complex to other transcriptional coactivators including GCN5 histone acetyltransferase. The p37 complex directly binds the enhancer and promoter regions of AIB1 and regulates its transcription by modulating histone modification that appears to impact on RNA Pol II pausing. In tamoxifen-resistant cells that overexpress AIB1, knockdown of SNAP1 further increased AIB1 level. Importantly, knockdown of BCAR2 and p37 reduced AIB1 level and significantly improved the tamoxifen sensitivity of these cells. Finally, we show that BCAR2 is up-regulated while SNAP1 is down-regulated in breast cancers. Together, this study shows that a properly balanced coactivator/corepressor function of the p37-SNAP1-BCAR2-HDAC complex is important for the normal expression of AIB1, and that deregulation of this balance is likely a major driving force of AIB1 overexpression in breast cancer. Supported by UC Cancer Center, Department of Cancer and Cell Biology, and Grants from ACS and NIH.
Abstracts:

Poster Presentations
EZH2 activation of AR/AR transcriptional signaling in metastatic prostate cancer

Jung Kim, Jonathan Zhao, Hongjian Jin, Longtao Wu, Jindan Yu

Robert H. Lurie Comprehensive Cancer Center, Department of Medicine – Hem/Onc, Northwestern University, Chicago, IL

Androgen receptor (AR), a family member of nuclear hormone receptors, plays an important role in prostate cancer progression through activation of oncogenes, such as PSA and TMPRSS2. Enhancer of Zeste homolog 2 (EZH2) is a histone methyltransferase that is highly overexpressed in metastatic prostate cancer patients and methylate tumor suppressors at histone3 lysine27 (H3K27) to promote tumor progression. Previously, our lab have identified that EZH2 regulates AR in prostate cancer. However, the molecular mechanism of how EZH2 regulates AR was not identified. Therefore, in this study, we used LAPC4 as our model system to understand the molecular mechanism of how EZH2 regulates AR transcriptional activity. First, we identified miR-30e as an intermediate factor of EZH2 and AR using bioinformatics approach. Then, we used GSEA and molecular approaches to reveal that EZH2 regulation of AR is indeed through miR-30e. Here, our study shows that EZH2 inhibit miR-30e at the promoter region, therefore, inhibiting miR-30e to target AR at AR 3’UTR. This study provides an important link between EZH2 and AR, two proteins that plays an important role in prostate cancer progression.
Activation of nutritional sensor REDD1 is necessary for the development of steroid-induced cutaneous atrophy.

G. Baida 1, R. Lavker 1, P. Bhalla 1, T. Finkel 3, E. Lesovaya 4, K. Yuen 1, I. Budunova 1.

1- Department of Dermatology, Northwestern University, Chicago, IL, USA
2- N. Blokhin Cancer Research Center, RAMS, Moscow, Russia
3- CHOP, Philadelphia, Pennsylvania, USA

One of the major adverse effects of topical glucocorticoids is cutaneous atrophy. The molecular mechanisms underlying this phenomenon are poorly understood. To elucidate the mechanisms of epidermal hypoplasia, we used chronic topical treatment of mouse and human skin with glucocorticoids which resulted in marked epidermal thinning and depletion of the interfollicular keratinocyte population. We found that a single exposure or chronic treatment with glucocorticoids induced a strong increase in the expression of REDD1 (regulated in development and DNA damage response) at mRNA and protein levels in mouse and human epidermis. REDD1 is a nutritional sensor and a stress-inducible inhibitor of mTOR complex 1, critical for protein synthesis regulation. Accordingly, we also noted a strong decrease in phospho-mTOR S2448 expression in epidermis after exposure to glucocorticoids. REDD1 is a primary target gene for the glucocorticoid receptor (GR), whose expression is regulated via GR binding sites in the REDD1 promoter. We observed that glucocorticoids strongly induced expression of TXNIP (thioredoxin-interacting protein) that binds to and stabilizes REDD1 protein. We also observed down regulation of conservative miRNA-221 that regulates REDD1 expression. Most importantly, REDD1 knockout animals appeared resistant to epidermal atrophy induced by glucocorticoids. Finally, we revealed an excellent correlation between REDD1 induction in skin and individual animal sensitivity to adverse effects of topical steroids. Collectively, our findings reveal a novel mechanism of glucocorticoid-induced skin atrophy and link glucocorticoids to the control of protein synthesis in keratinocytes via mTOR regulation. In addition, our findings are relevant to the development of selective GR activators that have safer therapeutic profiles than classical steroids. Supported by Supported by RO1CA118890 (to I.B. and R.L.).
Aminoflavone and the aryl hydrocarbon receptor: potential therapeutic applications in breast cancer

A.M. Brinkman\textsuperscript{1,2}, W. Xu\textsuperscript{1,2}

\textsuperscript{1} McArdle Laboratory for Cancer Research, University of Wisconsin–Madison, Madison, WI, \textsuperscript{2} Molecular and Environmental Toxicology Center, University of Wisconsin-Madison, Madison, WI

Several studies have suggested that the aryl hydrocarbon receptor (AhR) may be a potential therapeutic target for numerous human diseases, including estrogen receptor (ER) positive breast cancer. While ER negative breast cancer cell lines also express AhR, the antiproliferative activity of AhR ligands against this type of breast cancer has not been thoroughly investigated. Aminoflavone (AF), a ligand of the AhR that is structurally related to phytochemicals called flavonoids, is currently in clinical trials for the treatment of solid tumors. It has been hypothesized that presence of ER is a predictor for antiproliferative activity of AF, with ER positive cell lines being sensitive to the drug, and ER negative cell lines being resistant. In addition, cellular localization of the AhR is thought to be related to the effectiveness of AF, with cytoplasmic AhR being correlated to antiproliferative activity. Here, we show that AF exhibits antiproliferative activity in two ER negative breast cancer cell lines, MDA MB 468 and Cal51. This work suggests that presence of ER may not be the sole determinant of AF-mediated antiproliferative activity in breast cancer cells. Supported by NIEHS Predoctoral Training Grant T32 ES007015 and MRC-SOT Young Investigator Award.
Retinoic acid (RA) is crucial for prostate development and maintenance of differentiation in mature prostate. Aberrant RA signaling has been reported as a precursor lesion to many cancers including prostate cancer. Despite of the importance of RA in prostate development and pathogenesis, the mechanism by which RA regulates prostate cell proliferation remains unknown.

Here, we report in human prostate epithelial cells that stably express human androgen receptor (HPr-1AR), RA elicited biphasic effects on cell proliferation, including short-term stimulation and long-term inhibition of proliferation. By contrast, RA stimulated the proliferation of LNCaP prostate cancer cells. Hence, the effect of RA on prostate proliferation is cell type-specific. As RA could activate both RARs and PPARD, we treated cells with ligands that selectively activate RARs or PPARD. Remarkably, RARs were the primary mediator of RA regulated proliferation in HPr-1AR, whereas RARs and PPARD modulated LNCaP cell proliferation. Notably, in HPr-1AR, RA induced hallmarks of autophagy, including cell vacuolization and LC3-II conversion, suggesting that the mechanism for inhibition of cell proliferation by long-term RA treatment may involve autophagy.

As the proliferation of HPr-1AR and LNCaP are both responsive to androgen, we assessed the effect of androgen and RA co-treatment. In HPr-1AR, DHT inhibited proliferation, whereas RA stimulated proliferation; and the co-treatment returned proliferation to the control level. In LNCaP, treatment with either DHT or RA stimulated cell proliferation; however, co-treatment with DHT and RA attenuated the stimulatory effect of the individual treatments. These findings indicate that RA and androgen signaling can mutually inhibit each other. A possible mechanism may involve mutual down-regulation of receptor expression. Overall, this study may help define the role of RA in prostate proliferation and possibly, reveal more targets for cell proliferation control.
Poster #5

Investigating the in vivo effects of estrogen receptor heterodimer inducing phytoestrogens

Carlos Coriano\textsuperscript{1,2}, Chelsie Kohns\textsuperscript{1}, Zhisheng Jiang\textsuperscript{2}, Jill Kolesar\textsuperscript{3}, James Shull\textsuperscript{1} and Wei Xu\textsuperscript{1,2}

\textsuperscript{1}Molecular & Environmental Toxicology, \textsuperscript{2}McArdle Laboratory for Cancer Research, \textsuperscript{3}Division of Pharmaceutical Sciences, University of Wisconsin-Madison.

Estrogen plays an important role in the normal development and growth of the mammary gland. The binding of 17\textbeta-estradiol (E2) and other estrogenic compounds to the estrogen receptors (ER) leads to its dimerization and consequent genomic transcription and cascade of biological activities. ER\textalpha and ER\textbeta have been shown to exhibit a “Ying and Yang” relationship in regulating breast cancer cell growth: ER\textalpha promotes cell growth while ER\textbeta inhibits it. At the receptor level, ER\textbeta appears to negatively regulate the proliferative role of ER\textalpha through the formation of ER\textalpha/ER\textbeta heterodimers. The objective of this study is to identify natural estrogenic compounds which promote the formation of ER\textalpha/ER\textbeta heterodimers, thereby activating ER\textbeta while dampening ER\textalpha activity, which might have a protective effect in breast cancer. We screened 37 phytoestrogenic compounds from four flavonoid subclasses in a two-step screening process that include activation of ER gene transcription using an ER-dependent reporter gene assay and dimerization using the Bioluminescent Resonance Energy Transfer (BRET) assay and have proposed to test the cancer preventive effects of compounds that induce the ER\textalpha/ER\textbeta heterodimer in E2-induced breast cancer model of ACI rats. Supported by NIH-R01 CA125387
Effect of flaxseed on estrogen signaling and metabolism in ovarian cancer using the laying hen model.

Anushka Dikshit and Dale Buchanan Hales

Department of Physiology, Southern Illinois University School of Medicine, Carbondale, IL.

Anti-oncogenic properties of flaxseed are predominantly due to two of its major components, the omega-3-fattyacids and the lignan Secoisolaricirescinol diglucoside (SDG) that is converted to enterodiol (ED) and enterolactone (EL) by the bacterial flora in the gut. ED and EL have weak anti-estrogenic/estrogenic properties. Cyp3A4, Cyp1B1 and Cyp1A1 act on 17β estradiol (E2) to form 16-hydroxy E2 (16-OHE), 4-hydroxy E2 (4-OHE) and 2-hydroxy E2 (2-OHE), respectively. 4-OHE and 16-OHE on oxidation become genotoxic and carcinogenic. Low 2OHE/16OHE ratio in urine or serum is correlated to increased risk of cancer. Cyp19 (aromatase) catalyzes the last step in E2 synthesis.

The objective of our study was to understand the effect of flaxseed on estrogen signaling and metabolism in normal and cancerous ovaries of the hen, which is the only animal model that develops spontaneous epithelial ovarian cancer that mimics the human disease. In Study # 1, chickens were fed either 10% flaxseed or control diet for five years, while in Study # 2, chickens were fed with doses of 0%, 5%, 10% and 15% flaxseed for four months. Ovaries were classified as either normal or cancerous based on gross pathology and histology. mRNA levels were measured using qPCR while protein levels were analyzed using IHC and western blotting. ERα, ERβ, Cyp3A4 and Cyp1B1 mRNA levels were down regulated in flax-fed hens over time and with increased flax dosage. Cyp19 expression was however up regulated in the flax normal and flax cancer groups when compared to control. Serum analysis (Estramet 2/16 kit) indicated that the 2OHE/16OHE ratio was significantly increased in the flax-fed birds.

In conclusion, flax diet decreases expression of estrogen receptors (ERα & ERβ) and estrogen-metabolizing enzymes (Cyp3A4, Cyp1B1) in turn leading to decreased estrogen signaling and lower levels of toxic estrogen metabolites.
[Supported by NIH AT00408, AT005295 and AICR 06-A043]
Detecting the DNA Methylation Fingerprint in Endometriosis

Matthew T. Dyson\textsuperscript{1}, Damian Roqueiro\textsuperscript{2}, Toshiyuki Kakinuma\textsuperscript{1}, Diana Monsivais\textsuperscript{1}, Serdar E. Bulun\textsuperscript{1}

\textsuperscript{1}Division of Reproductive Biology Research, Dept. Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL
\textsuperscript{2}Laboratory of Computational Functional Genomics, Dept. Bioengineering, University of Illinois at Chicago, Chicago, IL

Endometriosis causes infertility, adhesions and chronic pain in approximately 10\% of reproductively-aged women due to the extrauterine growth of endometrial-like cells. The etiology of the disease is enigmatic; however, DNA methylation is predicted to be an underlying problem, since such epigenetic changes can be both mutable and heritable. Furthermore, changes in DNA methylation have been correlated to the altered expression of several genes implicated in the pathology of endometriosis. We hypothesize that altered DNA methylation in endometriotic stromal cells affects their gene expression patterns and response to steroid hormone signaling. Normal endometrial stromal cells (HESC) were obtained from 6 patients without endometriosis undergoing hysterectomy for benign complications. Endometriotic stromal cells (OSIS) were obtained from 6 patients having surgical removal of ovarian endometriotic cysts. All patients were premenopausal, and underwent surgery during the proliferative phase of their menstrual cycle having received no preoperative hormonal therapy. The gene expression results (HT12v4, Illumina) identified 982 differentially regulated genes. The methylation array (HM450, Illumina) revealed 33,260 differentially methylated CpGs. Only 11\% of these mapped to canonical CpG islands, whereas more than 55\% were located in regions unassociated with any CpG island. Notably, 5113 of the differentially methylated CpGs were matched to genes also showing altered expression. Within this group, 3394 CpGs were hypermethylated in OSIS. Gene ontology analysis of the matched hypermethylated genes identified processes involved in urogenital and female sexual development, whereas hypomethylated genes were enriched in processes regulating cell migration and locomotion. The focused differences in methylation and gene expression provide insight into the molecular changes underlying endometriosis, and point us toward new genetic targets as we look to understand how it arises.
Long term consumption of flaxseed enriched diet decreased ovarian cancer incidence and PGE2 in hens

Erfan Eilati 1, Lurui Pan 1, Janice M. Bahr 2, Dale Buchanan Hales 1

1 Department of Physiology, Southern Illinois University - Carbondale, School of Medicine, Carbondale, IL, USA
2 Department of Animal Sciences, University of Illinois at Urbana-Champaign, Urbana, IL, USA

The association between chronic inflammation and cancer has been recognized for many years. Cyclooxygenase (COX) (PTGS) is the rate limiting enzyme in catalyzing the conversion of arachidonic acid to prostaglandins (PG) such as PGE2 which has been identified as a key pro-inflammatory molecule. COX has two isoforms, COX-1 and COX-2. The effect of environmental factors such as diet on cancer is clear. Excessive consumption of omega-6 (OM-6FA) fatty acids in western diet has been linked to malignancies. Modification of substrate availability for COX enzymes and reducing the expression/activity of them to affect PGs by using omega-3 (OM-3FA) is an alternative approach to prevent or treat cancer. Flaxseed is the richest plant source of one kind of OM-3FA, alpha-linolenic acid (ALA). Ovarian cancer is the fifth leading cause of cancer death among women and the most lethal gynecological malignancy. Hens spontaneously develop ovarian adenocarcinomas that are similar in histological appearance to human ovarian carcinomas and share similar symptoms of the disease thus the laying hen is the only accessible animal model that faithfully recapitulates human ovarian cancer. Previously we have shown that consumption of 10% flaxseed diet for a year decreases the severity of ovarian cancer in hens; in this experiment we aimed to examine the long term effect of 10% flaxseed on ovarian cancer incidence and inflammatory factors.

900 six month old white Leghorn hens were divided into two groups and fed either control or flax diet for 5 years. Twenty hens from each group were necropsied every 6 months. The incidence of ovarian cancer was determined by gross pathology and histology. COX-1 and COX-2 and PGE2 receptor-subtype 4 (EP4) (PTGER4) mRNA and protein were measured in ovarian tissues using q-PCR and western blot, respectively. PGE2 amounts in ovarian tissues were measured using ELISA. No ovarian cancer was detected in hens aged 1, 1.5 and 2 years. Starting at 2.5 years hens were first observed with ovarian cancer with incidence increasing with age. Similarly, very little PGE2 was detected in ovaries of 1, 1.5 and 2 years old hens but in correlation with cancer incidence, PGE2 concentration increased in ovaries of hens aged 2.5 years and older. Results indicated lower incidence of ovarian cancer in flax fed hens compared to control. Interestingly, flaxseed caused a significant reduction in PGE2 concentration in ovaries of 2.5 and 3.5 years old hens which was correlated with decreased incidence of ovarian cancer. Expression of both COX-1 protein and mRNA in flax fed hens was slightly less than control fed hens; however, it was not statistically significant. Similar results were observed for COX-2. There was no difference in expression of EP4 protein and mRNA between two groups. The present study provides the first insight into effect of flaxseed on
expression of COX-1, COX-2, EP4 and concentration of PGE2 and ovarian cancer incidence in hens. The current study demonstrated that decreasing PGE2 in ovaries of hens may have a crucial role in mechanism of action of flaxseed against incidence and severity of ovarian malignancies. These finding may provide new insight into the ability of flaxseed and OM-3FA to decrease ovarian cancer may establish the foundation for clinical studies to test the efficacy of dietary intervention with flaxseed for the prevention and suppression of ovarian cancer in women. [Supported by NIH AT00408, AT005295 and AICR 06-A043]
Poster #9

**Growth Hormone Potentiates the Proliferative Effect of Estrogen in Breast Cancer Cells in an AKT-dependent Manner.**

Lamiaa El-Shennawy¹, Dana Felice², Steve Swanson³, Terry Unterman⁴ and Jonna Frasor²

¹Department of Biopharmaceutical Sciences; ²Department of Physiology and Biophysics; ³Department of Medicinal Chemistry and Pharmacognosy; ⁴Department of Medicine; University of Illinois at Chicago, Chicago, IL 60612

Approximately 70% of breast tumors express estrogen receptor (ER) and can be targeted with endocrine therapies. However, up to 50% of patients with ER+ tumors will fail to respond to these drugs. Due to the importance of ER, it is imperative to understand how different hormones and growth factors affect its activity. Growth hormone (GH), which is produced not only by the pituitary but is also expressed in breast tumors, is significantly associated with lymph node metastasis, tumor stage, and proliferative index, as well as worse relapse-free and overall survival. Recent work from our lab has shown that GH acting through Jak2, EGFR and ERK can affect ER+ breast cancer cells. In this study we investigated the effect of GH on the serine/threonine kinase AKT because activation of this pathway is known to promote tumorigenesis and is associated with endocrine therapy failure. The Spontaneous Dwarf Rat (SDR) model lacks endogenous GH and requires both GH and E2 to develop chemically induced mammary tumors. We found a high level of AKT activation in tumors of SDR treated with GH. A corresponding down-regulation of the AKT target, FoxO3a, was also observed. Similarly, we found that GH treated T47D breast cancer cells also have activated AKT. Co-treatment of T47D cells with both estradiol (E2) and GH led to significantly greater AKT activation compared to GH alone. Proliferation of T47D cells was also increased by the combination of GH+E2 compared to E2 or GH alone. Blocking AKT activation with the chemical inhibitor LY-294002 abolished the effect of both E2 and GH on T47D proliferation. Our data suggest that AKT may mediate the effect of GH in ER+ breast cancer cells. Further investigation into the nature of crosstalk between GH and E2 is underway with the goal of using this knowledge to develop novel therapies for breast cancer patients. Supported by the American Cancer Society (JF) and the International Fulbright Science and Technology Award (LE).
The nuclear and extranuclear actions of the nuclear hormone receptor estrogen receptor alpha (ERα) depend on the formation of homodimers. Tamoxifen and raloxifene, termed selective estrogen receptor modulators (SERMs), are part of a growing family of drugs approved to treat or reduce the risk of ERα-positive breast cancers. Classical SERMs act by competitively binding to the ER α/β ligand-binding domain (LBD). Unfortunately, SERMs have undesirable side effects and reduced efficacy in women with progressive ERα-positive disease. Here, both computational design and library screening approaches are being used to identify and develop small molecules that selectively bind to and disrupt the ERα LBD homodimer interface. A simple, high-throughput fluorescence assay will probe for the exposure of hydrophobic residues buried within the dimer interface. The resulting small molecule hits will stabilize ERα-LBD conformations that preclude homodimer formation. As such, these molecules will functionally disrupt ERα signaling independent of classical SERM binding to the ligand binding pocket. Finally, comprehensive biophysical, structural and tissue culture investigations will guide compound redesign efforts and probe their pharmacological activity in a biologically relevant setting. Ultimately, our goal is to develop the best possible small molecule therapeutics to treat or reduce the risk of ERα-positive breast cancers. Supported by the Ludwig Center for Metastasis Research.
Puriﬁcation & characterization of full-length Rev-erbβ, a heme-responsive nuclear receptor

Nirupama Gupta, Eric Carter, Janet Rhee and Stephen W Ragsdale

Department of Biochemistry, University of Michigan Medical School, Ann Arbor, MI.

Rev-erbs (Rev-erbα and Rev-erbβ) are nuclear receptors that regulate the expression of a broad spectrum of genes involved in metabolism, circadian cycle, and proinflammatory responses. Rev-erbs bind heme at their ligand-binding domains (LBD), which leads to the recruitment of the nuclear receptor corepressor (NCoR): histone deacetylase complex and repression of target gene expression.

Very few full-length nuclear receptors have been expressed and puriﬁed in E. coli. Here we report the puriﬁcation and initial biochemical analysis of FLRev-erbβ heterologously produced in E. coli. FLRev-erbβ shows high afﬁnity binding to DNA and heme with dissociation constants (Kd) of 50 nM and 4 nM, respectively. Interestingly, the protein demonstrates redox-dependent binding to DNA and heme, similar to what we have reported for the LBD. The oxidized protein does not bind DNA and has lower afﬁnity for heme. We propose that oxidation of Rev-erb thiols cause conformational changes that decrease the afﬁnity for heme and DNA. Indeed, we observe quaternary structural changes in the protein upon oxidation. We measured the redox potential of the switch to be -166 mV, indicating that Rev-erbβ is fully reduced under normoxia, but may be prone to aggregation under oxidative stress conditions. In vivo, the protein could undergo re-reduction by thioredoxin, as has been proposed for the glucocorticoid receptor. In addition to DNA and heme, we have shown that both FLRev-erbβ and the LBD bind a NCoR-derived peptide, ID1 with high afﬁnity (Kd ~11 nM). FLRev-erbβ complexed with oligonucleotides containing RORE/Rev-DR2 elements exhibits the same afﬁnity for ID1 as the free protein, indicating autonomous functionality for the individual subunits. In summary, we have studied DNA, heme, and corepressor binding properties of FLRev-erbβ that matches well with previous biochemical analyses of the individual domains.

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Hepatocyte levels of nuclear Akt and 8-hydroxydeoxyguanosine (8-OHdG), a marker for oxidative damage, are positively correlated, and nuclear Akt expression is higher in areas of dysplastic than in non-dysplastic cirrhotic liver tissue.

Grace Guzman1, Bryan Rea2, Ada Quintana1, Rohini Chennuri1, Nissim Hay3

1Pathology, 2College of Medicine, 3Biochemistry and Molecular Genetics, University of Illinois, Chicago, IL, USA

Background: Accumulation of ROS in human tissues can cause damage to cell DNA and proteins. Animal studies implicate Akt in the development of hepatocellular carcinoma and show that nuclear (n) Akt phosphorylates FOXO transcription factors and excludes them from the nucleus, thereby inhibiting FOXO transcriptional activity (Hay, Biochim Biophys Acta 2011; Nogueira, Cancer Cell 2008). The inhibition of FOXO by nAkt reduces levels of ROS scavengers such as MnSOD (SOD2), Catalase, and Sestrin3, leading to increased levels of ROS. Hence, we hypothesized a direct relationship between nAkt and 8-OHdG levels. Aims: To assess levels of nAkt and 8-OHdG, and to evaluate for an association between nAkt and 8-OHdG expression in human liver tissues including normal controls, and non-dysplastic and dysplastic cirrhotic liver tissue. Methods: We designed and analyzed a progression liver tissue array derived from 100 liver explant specimens and 8 normal livers for the expression of nAkt and and 8-OHdG by standard immunohistochemistry. Staining was quantified by image analysis. Dysplasia was defined as hepatocytes showing nuclear features such as hyperchromasia, atypia, or variable size, arranged in groups or dispersed, and maintained the usual one to two cell thick plates (Anthony, J of Clin Path 1973). Nonparametric statistical tests (Mann-Whitney and Wilcoxon) and correlation coefficients (Spearman Rho test) were employed using SPSS Statistics 17.0. Results: Subjects were 62% female; mean age: 50 +/- 9 years; ethnic distribution: 25% African American, 37% non-Hispanic white, 32 % Hispanic, and 6% other ethnicity; cause of liver disease: viral hepatitis (54%), alcohol (23%); NASH (11%); and other etiology (12%). Expression of nAkt was significantly greater in areas of dysplasia than in cirrhotic liver tissue (p=0.016). nAkt expression trended toward being higher in cirrhosis (p=0.062) than in controls and was significantly greater in dysplasia (p=0.026) compared to normal control tissue. 8-OHdG expression was not significantly higher in cirrhosis than controls (p=0.164) and trended toward being greater in dysplastic cirrhotic tissue (p=0.057) compared to normal control tissue. There were significant positive correlations between nAkt and 8-OHdG expression in non-dysplastic cirrhotic (r=0.65, p<0.001), and in dysplastic cirrhotic (r=0.55, p<0.001) liver tissue.

Conclusion: The current study shows a direct relationship between nuclear (n) Akt and 8-OHdG levels in non-dysplastic and dysplastic cirrhotic liver tissue, indicating that nAkt
expression is associated with increased levels of oxidative damage in the liver. nAkt expression was greater in dysplastic cirrhotic compared to non-dysplastic cirrhotic and normal liver tissue, implicating nuclear Akt in precancerous changes in chronic liver disease in humans.

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Comparison of the chemopreventive potential of two licorice species with hops

Atieh Hajirahimkhan, Birgit M. Dietz, Huali Dong, Charlotte Simmler, Shao-Nong Chen, Dejan Nikolic, Guido F. Pauli, Richard B. van Breemen, Judy L. Bolton

UIC/NIH Center for Botanical Dietary Supplements Research, Department of Medicinal Chemistry and Pharmacognosy, College of Pharmacy, University of Illinois at Chicago, Chicago, IL.

Objectives: Licorice has chemopreventive potential through activation of detoxification enzymes.

The Women’s Health Initiative demonstrated increased risk of breast cancer associated with hormone therapy used for the alleviation of menopausal symptoms. Therefore, many women turn to botanical dietary supplements as natural alternatives, without having enough evidence about their safety and efficacy. Hops (Humulus lupulus) and licorice (Glycyrrhiza glabra and Glycyrrhiza uralensis) are common components of menopausal dietary formulations. Our previous studies have demonstrated chemopreventive potential for hops through activation of detoxification pathways in cultured mammary and hepatic cells as well as in female Sprague Dawley rats. Our in vitro observations have shown that similar to hops, both licorice species can enhance induction of NAD(P)H: quinone oxidoreductase 1 (NQO1) in murine hepatoma cells and both plants induced NQO1 and glutathione S-transferase P1 in ERα negative human breast epithelial cells (MCF10A), likely through activation of Nrf2 signaling pathway. While the estrogenic compound 8-prenylnaringenin from hops did not have NQO1 inducing effects, its precursor chalcone, xanthohumol, showed strong chemopreventive potential. Interestingly, liquiritigenin, the estrogenic compound from licorice and its precursor chalcone, isoliquiritigenin, both induced NQO1 in murine hepatoma cells. The in vitro studies with licorice in hepatic and mammary cells and the observed similarities with hops suggest chemopreventive potential for licorice and warrants animal studies to analyze its detoxification enzyme inducing effects in vivo. Support was provided by NIH grant P50 AT00155 provided to the UIC/NIH Center for Botanical Dietary Supplements Research.
Mechanisms and molecular targets of phytoestrogens in breast cancer in vivo

T.T James¹², Y. Wang¹, E. Powell¹, W. Xu¹²,

McArdle Laboratory for Cancer Research¹, Molecular & Environmental Toxicology Center²,
University of Wisconsin-Madison, Madison, WI.

Estrogens play essential roles in regulation of mammary gland development and morphogenesis, maturation of uterus and ovaries, and bone homeostasis. These functions are mediated by two subtypes of estrogen receptors, ERα and ERβ, which function in dimer forms (i.e. ERα, β homodimers and ERα/β heterodimers). The ratio of ERα: ERβ increase during tumorigenesis. Although ERα is the main target for breast cancer therapy, ERβ is neither a prognosis marker nor a therapeutic target. Because the current ER targeting drugs (e.g. fulvestrant) non-selectively inhibit both forms of ERs, better strategies are needed to utilize the anti-proliferative effects of ERβ. Approximately 60% of breast tumors express both ERα and ERβ, and it has recently been shown by our laboratory that both receptors can be found in the same cell. Additionally, we recently demonstrated that like ERβ homodimers, ERα/β heterodimers are anti-proliferative in breast cancer cells, suggesting that ligands selective for ERβ dimerization may be useful for cancer treatment. Our lab pioneered in developing a BRET1 (Bioluminescence Resonance Energy Transfer) assay to monitor ERα/β heterodimer in live cells and identified phytoestrogens (e.g. cosmosin) exhibiting heterodimer specificity. However, BRET1 is not suitable for determining ER dimerization in vivo. To overcome this limitation, a novel BRET3 assay will be developed in this project. In analogy to BRET1, BRET3 also requires fusion of ER to a fluorescent protein. However, in BRET3 this protein is more red-shifted resulting in less absorption by tissues and more reflection of light, making it suitable for in vivo imaging. We believe that phytoestrogens displaying ERα/β heterodimer specificity in vivo will inhibit the progression of breast cancers which have both ERs expressed, allowing alternative treatments for breast cancers that failed endocrine therapy. Supported by NIEHS Training Grant T32 ES007015.
Effects of pregnancy-specific hormones on cytochrome P450 (CYP) expression in human hepatocytes.

Jin Kyung Lee¹, Hye Jin Chung¹, Xian Pan¹, Liam Fischer¹, Magnus Ingelman-Sundberg², and Hyunyoung Jeong¹

¹ Department of Pharmacy Practice, College of Pharmacy, University of Illinois at Chicago, Chicago, IL 60612, USA; ² Section of Pharmacogenetics, Department of Physiology and Pharmacology, Karolinska Institutet, SE-171 77 Stockholm, Sweden.

Pregnancy alters hepatic drug metabolism, but the responsible factors and underlying mechanism remain to be identified. Physiological changes accompanying pregnancy are potentially responsible for the changes, such as rising concentrations of pregnancy-specific somatotropins: prolactin (PRL), placental lactogen (PL), and growth hormone variant (GH-v). Whether these hormones influence hepatic drug metabolism in humans is unknown. In this study, we evaluated the effects of PRL, PL, and GH-v on expression of major hepatic cytochrome P450 (CYP) enzymes. To this end, primary human hepatocytes (from 3 different female donors) were treated with vehicle, PRL (150 ng/mL), PL (6 μg/mL), or GH-v (20 ng/mL) for 72 hr, and mRNA expression levels of CYP 1A2, 2A6, 2B6, 2C9, 2C19, 2D6, 2E1, 3A4, and 3A5 were determined by quantitative real-time PCR. PL increased mRNA expression level of CYP2E1 (2.8 to 5.1-fold), CYP3A4 (1.4 to 4.7-fold), and CYP3A5 (2.5- to 4.5-fold), but PRL and GH-v had no reproducible effect on expression of all CYP isoforms tested. The induction of CYP2E1 mRNA by PL was concentration-dependent. PL increased CYP2E1 protein level, thereby leading to 74% increases in chlorzoxazone 6-hydroxylation activity. Follow-up mechanism studies revealed that signal transducer and activator of transcription 5 (STAT5) was in part involved in the transcriptional regulation of CYP2E1 by PL. Taken together, these results indicate that pregnancy-specific somatotropin, PL increases CYP2E1 expression, and this could potentially lead to altered CYP2E1-mediated drug metabolism during pregnancy, resulting in differential therapeutic efficacy and/or toxicity in pregnant women.

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Estrogen potentiates NFkB activity to increase PHLDA1 and cancer stem cell phenotype in ER+ breast cancers cells.

Irida Kastrati and Jonna Frasor

Department of Physiology and Biophysics, College of Medicine, University of Illinois at Chicago, Chicago, IL

Activation of NFkB in a subset of ER+ cancers is associated with poor prognosis and resistance to endocrine and chemo therapy. Previous work from our lab showed that inflammatory cytokines and the NFkB pathway may contribute to ER+ luminal B cancers by acting with ER to induce a specific set of genes associated with cell survival. Of these genes, PHLDA1 (pleckstrin homology-like domain, family A, member 1) is of interest because recent reports suggest it is an epithelial stem cell marker contributing to intestinal tumorigenesis. We hypothesized that the aggressive features of luminal B cancers may be due to cooperative crosstalk between ER and NFkB leading to upregulation of PHLDA1 and increased CSC properties.

In ER+ breast cancer cells we find that PHLDA1 expression is increased by pro-inflammatory cytokines, such as TNFa or IL-1b, in an NFkB-dependent manner. The addition of estradiol (E2) further enhances PHLDA1 expression in an ER-dependent manner. HDAC inhibitors suppress the effect of E2 on PHLDA1, suggesting that ER recruits an HDAC to potentiate NFkB activity.

To understand if ER and NFkB promote CSC properties we treated MCF-7 cells grown as 3D mammospheres, a typical assay for in vitro CSCs. We found that SERMs, the ER-antagonist ICI182,780, and several NFkB pathway inhibitors reduce mammosphere growth suggesting that both pathways are active and essential for mammosphere growth of ER+ cells.

Interestingly, PHLDA1 is highly expressed in multiple ER+ breast cancer cells grown as mammospheres compared to standard 2D adherent culture. Silencing of PHLDA1 resulted in decreased overall number and average size of mammospheres, indicating that PHLDA1 could be important in the CSC phenotype of ER+ cells. Elucidating PHLDA1 function and regulation could uncover a novel target for luminal B breast cancers and address the unmeet need of endocrine-resistance in ER+ tumors with activated NFkB.

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Exploring mechanisms of EAF2 action – from RNAi screen in C. elegans to mammalian analysis

A.L. Keener\textsuperscript{1,2}, L. Cai\textsuperscript{2}, J. Ai\textsuperscript{2}, Z. Wang\textsuperscript{1,2}

\textsuperscript{1}Department of Pharmacology and Chemical Biology, University of Pittsburgh School of Medicine, Pittsburgh PA  
\textsuperscript{2}Department of Urology, University of Pittsburgh Cancer Institute, University of Pittsburgh Medical Center, Pittsburgh, PA

EAF2 (ELL-associated factor 2) (also known as androgen up-regulated 19 or U19) is a RNA polymerase II transcription elongation factor. EAF2 positively regulates Eleven-Nineteen Lysine Rich Leukemia Factor (ELL), another RNA polymerase II transcription elongation factor. EAF2 is a tumor suppressor and it is up-regulated by the androgen receptor in the normal prostate. Over-expression of EAF2 in cancer cell lines causes apoptosis and when EAF2 is knocked down in mice, mice develop leukemia, liver carcinoma, lung adenocarcinoma, and prostate carcinoma. The \textit{C. elegans} ortholog to EAF2 is eaf-1. When eaf-1 is knocked out in \textit{C. elegans}, the resulting worms have reduced fertility, cuticle malformation, and have the phenotype known as “dumpy,” i.e. are smaller than WT worms. The eaf-1KO \textit{C. elegans} worms were used to screen for enhancers, genes that make a known phenotype worse. Knockdown of the gene pha-4 was found to render the eaf-1KO worms sterile. The mammalian ortholog to pha-4 is FOXA1 (Forkhead Box A1), a pioneer factor for the androgen receptor. FOXA1 is required for normal differentiation of prostatic epithelial cells. We hypothesized that FOXA1 is essential for the regulation of EAF2. Co-immunoprecipitations (Co-IPs) were performed using tagged FOXA1 and EAF2 to test if EAF2 and FOXA1 bind. There is preliminary evidence that FOXA1 is in a complex with EAF2. Cycloheximide assays were also performed to test if FOXA1 affects EAF2 protein levels. EAF2 levels are reduced in the presence of FOXA1, but FOXA1 does not affect the degradation rate of EAF2. More work is needed to verify these results.
The structure and functions of the glucocorticoid receptor’s activation function-1 domain

S.H. Khan, J.A. Arnott, L. Jun, R. Kumar

Department of Basic Sciences, The Commonwealth Medical College, Scranton, PA-18509, USA.

Most of our understanding of the two transcriptional activation (AF) domains (AF1 and AF2) of steroid receptors (SRs) is with AF2, located within the globular ligand binding domain (LBD) composed of a hydrophobic pocket. In response to conformational changes induced by hormone, AF2 recognizes LXXLL motifs of coregulatory proteins such as the p160 family of steroid receptor coactivators (SRCs). Despite the fact that AF1, located in the N-terminal domain (NTD), constitutes the major transcriptional activity of many SRs, little is known about its mechanism of activation. This deficiency is due to the fact that the AF1 is composed largely of intrinsically disordered polypeptide (IDP). IDPs including SRs’ AF1 undergo a disorder-order transition upon interaction with target molecules via a “coupled folding and binding process” resulting into stabilization of an active structural conformation. However, the molecular and structural basis for these interactions and mechanisms of AF1 activation remains poorly defined. Our biophysical/structural data show that when incubated in the presence of a naturally occurring osmolyte, trehalose, AF1 of the glucocorticoid receptor (GR) adopts a significantly higher helical structure with characteristics of native-like functional conformation. We further tested whether interaction of AF1 with a binding partner, TATA box binding protein (TBP) also induces such structure formation in AF1. Our data show that similar to trehalose, BP binding to AF1 also induces more helical content in AF1 at the expense of random coil conformation. Further, in this folded conformation, AF1’s interaction with SRC-1 is significantly enhanced. These results suggest that under physiological conditions, AF1 may be adopting such conformation(s) for its efficient interactions with specific coregulators, which may provide a mechanism by which SRs’ NTD/AF1 regulate transcriptional activity of AF1, information essential for improved therapeutic targeting of SRs.
Regulation of CEACAM1 by PPARα-dependent Mechanisms

Saja S. Khuder, Lucia Russo, Sadeesh Ramakrishnan, Terry Hinds, Qusai Y. Al-Share, Edwin Sanchez and Sonia M. Najjar

University of Toledo Medical College

The Carcinoembryonic Antigen-related Cell Adhesion Molecule (CEACAM1) is a transmembrane glycoprotein that regulates insulin action by promoting receptor-mediated insulin endocytosis and degradation, a key mechanism of insulin clearance, which occurs mostly in liver, but to a lower extent in kidney. Mice with null deletion of Ceacam1 exhibit impairment of insulin clearance and consequently, hyperinsulinemia, insulin resistance, hepatic steatosis and visceral obesity. Obese humans exhibit insulin resistance with associated decrease in CEACAM1 levels. High-fat feeding causes insulin resistance by reducing CEACAM1 before inflammation develops. Together with the finding that CEACAM1 is metabolically regulated; being markedly reduced at fasting and elevated in parallel to acute rise in insulin in response to refeeding, these data provided the impetus to investigate the mechanisms leading to decreased Ceacam1 expression in obesity, at fasting and in response to high-fat feeding. One of the shared common biochemical mechanisms between these conditions is elevated fatty acid (FA) entry to the liver to be cleared via β-oxidation. This is attained by activating the Peroxisome Proliferator Activated Receptor α (PPARα), a nuclear receptor that forms heterodimers with retinoid x receptor alpha (RXRα) to bind to DNA at sites of target genes. Our preliminary data strongly suggest that FA-induced PPARα activation results in direct transcriptional inhibition of Ceacam1. We have shown that PPARα activation by WY-14,643 selective PPARα agonist causes PPARα binding to Ceacam1 promoter in rat and mice hepatoma cells and in the liver of wild-type, but not PPARα knockout mice, fed a Wy-supplemented diet for 3 days. Deletion and block mutation analyses identified a main site of PPARα/RXRα binding to Ceacam1 promoter. Further studies are underway to investigate the mechanisms involved in Ceacam1 regulation. With CEACAM1 playing a key role in maintaining insulin action and fat metabolism, these studies can potentially identify CEACAM1 as a tractable drug target to combat metabolic insulin resistance, fatty liver disease and obesity. These studies have been supported by NIH grant: 2R01DK054254-09 to SM Najjar.
Androgen Receptor-Mediated Inhibition of Cell Proliferation in HPr-1AR Human Prostate Epithelial Cells

Young-Chae Kim, Congcong Chen and Eric C. Bolton

Department of Molecular and Integrative Physiology, University of Illinois at Urbana-Champaign, Urbana, IL.

The androgen dihydrotestosterone (DHT) is a crucial hormone for male development and prostate cancer progression. In addition, androgen receptor (AR) expression is often elevated in prostate cancer cells to maintain the expression of andromedins such as growth factor and survival factors that modulate cell cycle progression. Previously, we identified hundreds of androgen responsive genes (ARGs) in HPr-1AR. Our objective here was to determine the cellular role of three ARGs in the regulation of cell proliferation, and to investigate the mechanisms by which AR regulates their transcription. We examined the effects of androgens on cell proliferation and found that DHT inhibited cell proliferation. After 72 hours of DHT treatment, the doubling time had increased 2-fold or more. The mRNA transcripts of cell cycle regulators such as cyclin D1, cyclin D2 and Notch1, which are crucial for normal prostate epithelial cell proliferation, were androgen-repressed 3-14 fold by DHT treatment. The reductions in mRNA abundance for these cell cycle regulators was AR-dependent, as they did not occur in HPr-1 cells, which are AR-deficient cells. Remarkably, cyclin D1 transcripts were destabilized and exhibited an increased rate of turnover following androgen treatment, whereas cyclin D2 and Notch1 mRNA stability was unchanged by androgen, suggesting a mechanism involving transcriptional repression. In addition, the transcripts of Notch1 regulated genes Hes1 and Hey1 were reduced following DHT treatment. As the ARGs cyclin D1, cyclin D2, and Notch1 have been implicated in the regulation of cell proliferation, our results suggest that in HPr-1AR cells the AR-mediated inhibition of cell proliferation by androgens involves transcriptional repression and/or mRNA destabilization of one or more of these ARGs. Our results provide insight into the mechanisms of gene-specific transcriptional regulation operating within hormone-responsive gene networks.
Role of Krüppel-like Transcription Factors as Transcriptional Repressors in Cytochrome P450 2D6 Induction during Pregnancy

K.H. Koh¹, X. Pan², A-M. Yu³, H. Jeong¹,²

¹Department of Pharmacy Practice; ²Department of Biopharmaceutical Science, College of Pharmacy, University of Illinois at Chicago, Chicago, IL. ³Department of Pharmaceutical Sciences, University at Buffalo, The State University of New York, Buffalo, NY.

Cytochrome P450 2D6 (CYP2D6) is a clinically important drug-metabolizing enzyme that is responsible for metabolism of ~25% of marketed drugs. Clinical data indicate that pregnancy increases CYP2D6-mediated drug metabolism by 20-50%. The objective of this study is to elucidate the mechanisms underlying CYP2D6 induction during pregnancy. To this end, we used the CYP2D6-humanized (tg-CYP2D6) transgenic mouse model, where human CYP2D6 gene and its (2.5 kb) upstream regulatory region are inserted into the mouse genome. Previously, we showed that CYP2D6 mRNA, protein, and activity increased by 2.4-3.5 fold in tg-CYP2D6 mouse livers during pregnancy compared to the pre-pregnancy levels, using qRT-PCR, western blots, and microsomal phenotyping, respectively. Among the nine mouse homologs of CYP2D6 (Cyp2d9, 2d10, 2d11, 2d12, 2d13, 2d22, 2d26, 2d34, and 2d40), Cyp2d40 showed increased expression at term as compared to pre-pregnancy (by 6.08-fold) in both wild and tg-CYP2D6 mice. In silico analysis of CYP2D6 and Cyp2d promoters revealed that CYP2D6 and Cyp2d40 harbor binding sites for Krüppel-like transcription factors (KLFs) in their promoters, but the rest of Cyp2d genes do not. Results from electrophoretic mobility shift assay showed that expression and/or activities of certain KLFs (among 17 different members) decreased during pregnancy. QRT-PCR experiment showed relatively high mRNA expressions for Klf3, 9, 13, and 15 in the livers of nonpregnant mice. In HepG2 cells, transient transfection of KLF3, 9, or 13 significantly repressed CYP2D6 promoter activity, indicating that they may act as repressors in CYP2D6 expression. These results together suggest that pregnancy may decrease KLF expression and/or activity, hence, de-repressing CYP2D6 expression. This provides potential mechanistic insights to CYP2D6 induction during pregnancy.

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Development of MCF-7 biosensor cells expressing fluorescence-tagged, endogenous Estrogen Receptor-alpha

Jessica D. Lang¹, Cate E. Cavanagh¹, Mark E. Burkard², and Elaine T. Alarid¹

¹Department of Oncology, University of Wisconsin-Madison, ²Department of Medicine, University of Wisconsin-Madison

Hormone responsiveness in breast cancer is primarily determined by the expression of Estrogen Receptor-alpha (ER), yet resistance to targeting hormone therapies frequently occurs. The MCF-7 cell line has been used extensively as a model for hormone-sensitive breast cancer that recapitulates clinical observations. Current methods for measuring endogenous ER expression rely on population effects at distinct endpoints. However, the dynamics of each cell within a population provides additional information of response to various stimuli. Here we describe an MCF-7 cell system in which ER protein under endogenous transcriptional regulation can be measured in single cells in real-time. Using recombinant adeno-associated virus (rAAV) technology, the last exon of ESR1 was targeted to insert the mCherry gene as a fusion protein. Independent verification indicates that the transcriptional function of ER is not impaired by C-terminal fusions. Following infection of MCF-7 cells, over 200 clones expressing mCherry were obtained. Ongoing screens of individual clones for expression of ER-mCherry fusion transcripts have already identified clones expressing the targeted fusion. Site-specific integration and intact hormone response are also being validated. Imaging analysis protocols have been established to measure changes in protein expression by immunofluorescence, which can be easily adapted to real-time measurements in these targeted MCF-7 cells. Ultimately, mechanisms of ER signaling can be assessed on a single cell level, providing more information on expression dynamics such as synchronicity of responses within a population and periodicity of potential cycles in ER protein expression. Additionally, the utility of the ER-mCherry fusion will allow breast cancer cells to be used as “biosensor” cells for direct measurement of ER protein in response to stimuli in high-throughput capacity, such as drug and RNAi libraries. Supported by NIH grants CA159578 and T32 CA009135.
Hyperactive Akt signaling causes progestin resistance in endometrial cancer through the modulation of Progesterone Receptor B transcriptional function.

Irene Lee and J. Julie Kim.

Division of Reproductive Biology Research, Department of Obstetrics and Gynecology Labs, Northwestern University, Chicago, IL.

Progestin resistance presents the most significant challenge to improving fertility-sparing treatment in type I endometrial cancer. Currently, the response rates to conservative progestin therapy vary considerably and the molecular mechanisms behind progestin resistance are not well understood. We hypothesized that in PTEN mutated endometrial cancers, hyperactive Akt signaling alters Progesterone Receptor B (PRB) transcriptional activity, leading to impaired progestin responses in endometrial cancer. Throughout our studies, we utilized either the allosteric Akt inhibitor, MK-2206 (MK), or siRNA directed against Akt (siAkt), to inhibit Akt activity in Ishikawa endometrial cancer cells. In order to identify the specific PRB gene targets that are modulated by Akt signaling, we performed microarray gene expression analysis. Upon real-time PCR validation of target genes, we identified a subset of PRB target genes whose expression is dependent upon both Akt and PRB. Additionally, we hypothesized that partnership with the PR cofactor, FOXO1, may suggest the mechanism by which inhibition of Akt affects expression of PRB target genes. We overexpressed a constitutively active form of FOXO1 in PRB cells, and found that a subset of PRB target genes were upregulated similarly to Akt inhibition treatment. Additionally, we performed ChIP analysis on select PRB target gene regulatory regions and demonstrate that both PR and FOXO1 recruitment is increased following progestin (R5020) and MK combinatorial treatment. To determine the biological ramifications of combinatorial progestin and Akt inhibitor treatments, we performed cell proliferation assays in PRB stably transfected Ishikawa cells and found that the combination of R5020 and siAkt inhibited cell proliferation more than either agent alone. Taken together, these data suggest that Akt is sufficient to disrupt PRB transcriptional activity, and that inhibition of Akt may improve progestin responsiveness in endometrial cancer.

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Physiological concentrations of genistein reduced ER-β promoter methylation and increased ER-β expression in prostate cancer cells

Abeer M. Mahmoud, Wancai Yang, Maarten C. Bosland

Department of Pathology, University of Illinois at Chicago, Chicago, Illinois, USA

One of the most abundant and potent isoflavones in soy is genistein which has an estradiol like structure and high affinity to estrogen receptor-β (ER-β). It has been suggested that genistein exerts its anti-cancer effects through binding to ER-β which has anti-proliferative and pro-apoptotic effects in prostate cancer. ER-β expression is downregulated in localized prostate cancer with increasing grade from Prostatic Intraepithelial Neoplasia (PIN) through low to high Gleason grade. One of the mechanisms by which ER-β is suppressed in prostate cancer is promoter methylation, the extent of which correlates with the ER-β expression level which in turn may be linked to the degree of aggressiveness of prostate cancer. We examined the effects of physiological range of genistein concentrations (0.5- 10 μmol/L) on ER-β promoter methylation and ER-β expression in LNCaP, LAPC-4, and PC-3 prostate cancer cells that exhibit various basal levels of ER-β expression. We demonstrated for the first time that genistein caused a significant dose-dependent reduction in ER-β promoter methylation in LNCaP and LAPC-4 cells, using methylation specific PCR. There was a corresponding dose-related increase in ER-β mRNA and protein levels in these two cell lines assayed by quantitative RT-PCR and immunoblotting, respectively. However, ER-β expression levels and ER-β promoter methylation were not changed in PC-3 cells, which could be attributed to the low basal level of ER-β promoter methylation and the high basal level of ER-β expression in this cell line. These findings suggest that physiological concentrations of genistein is capable of reactivating tumor suppressor pathways by reversing ER-β promoter methylation with a subsequent restoration of the level of ER-β expression in prostate cancer cells that have higher basal levels of methylation. Supported in part by Grant No. CA116195
Respiratory Syncytial Virus (RSV) represses glucocorticoid receptor phosphorylation and function

J.I. Webster Marketon1,2, C. Burnsides1 and J. Corry1

1Division of Pulmonary, Allergy, Critical Care and Sleep Medicine, Department of Internal Medicine, 2The Institute of Behavioral Medicine Research, Wexner Medical Center at The Ohio State University, Columbus, OH.

Respiratory syncytial virus (RSV) is a common cause of bronchiolitis in infants. Although anti-inflammatory in nature, glucocorticoids have been shown to be ineffective in the treatment of RSV-induced bronchiolitis and wheezing. In addition, the effectiveness of glucocorticoids at inhibiting RSV-induced pro-inflammatory cytokine production in cell culture has been questioned. We have previously shown that RSV infection inhibits glucocorticoid induction of glucocorticoid receptor (GR)-regulated genes in both A549 and BEAS-2B cells and in primary small airway epithelial cells. This repression requires viral replication but does not require the NFκB pathway. RSV infection of A549 cells does not alter GR protein levels or GR nuclear translocation but does prevent GR binding to the promoter of glucocorticoid responsive genes. Our recent data shows that RSV infection of A549 cells also prevents glucocorticoid-mediated phosphorylation of GR at serine 211. Phosphorylation of this site has been associated with increased GR activity. RSV mediated suppression of glucocorticoid-induced GR phosphorylation could be reversed by a protein phosphatase inhibitor suggesting that RSV impairs GR phosphorylation and function through activation of a protein phosphatase. In addition, the RSV effects on GR function are not mediated by an autocrine factor produced by the RSV infected cells. Preliminary data suggests that the RSV nonstructural (NS) proteins mediate the effects of RSV infection on GR function. Understanding the mechanism by which glucocorticoid insensitivity occurs during RSV infection could provide a novel therapeutic approach to enhance the efficacy of glucocorticoids as a treatment of RSV-related disease.

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ERβ Regulates Genes that have Kinase and GTPase Functions and Enhance Cell Survival in Endometriosis

Diana Monsivais, Matthew Dyson, Antonia Navarro, Mary Ellen Pavone, Serdar E. Bulun.

Division of Reproductive Biology Research, Northwestern University, Chicago, IL

Endometriosis is an estrogen-dependent gynecological disease that affects 6-10% of women of reproductive age. It is a major cause of chronic pelvic pain and infertility, and poses a heavy financial burden on society, with annual estimates totaling $22 billion in the US alone. Methylation defects have been characterized in stromal cells derived from ovarian endometriotic lesions. In particular, a CpG island in the ESR2 promoter region is hypomethylated and contributes to a corresponding increase in ERβ mRNA and protein relative to the normal endometrium. The high local levels of 17β-Estradiol (E2) present in the endometriotic lesion milieu underscore the importance of elevated ERβ expression in endometriosis. However, the precise contribution of ERβ to endometriosis has not been fully characterized. In this study we show that ERβ transcriptionally regulates a subset of genes whose corresponding proteins possess intrinsic kinase or GTPase activity and contribute to endometriotic cell proliferation and survival. We correlated the differentially expressed genes from a microarray that compared normal endometrial and endometriotic stromal cells with previously identified ERβ genomic targets from ChIP-on-ChIP and ChIP-Seq experiments. Using this strategy, we found 70 differentially expressed genes in endometriosis that are potentially regulated by ERβ, 42 of which encode phosphoproteins. We validated that two of these genes are in fact ERβ genomic targets by showing 1) that their transcript and protein levels increase in response to E_2, 2) that their expression decreases after ERβ siRNA knockdown, and 3) that ERβ is enriched at the promoter regions of these two genes. Furthermore, we showed that these two genes, ras-like and estrogen regulated growth inhibitor (RERG) and serum and glucocorticoid-regulated kinase-1 (SGK-1), contribute to cell proliferation and apoptosis in endometriotic stromal cells. Our results demonstrate that in endometriosis, ERβ regulates a novel and important network of genes with intrinsic kinase and GTPase activity that control cell fate. Moreover, our study underscores the contribution of an altered nuclear receptor to endometriosis and poses ERβ as a potential drug target for this debilitating disease.

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Poster #27

Novel steroid-conjugated contrast agents for molecular characterization of hormone-dependent diseases.

Georgette Moyle-Heyrman¹, Taryn R. Townsend², Preeti A. Sukerkar², Keith MacRenaris², Thomas J. Meade², Joanna E. Burdette¹

¹Department of Medicinal Chemistry and Pharmacognosy, University of Illinois at Chicago, Chicago, IL
²Department of Chemistry, Department of Molecular Biosciences, Department of Neurobiology and Physiology, and Department of Radiology, Northwestern University, Evanston, IL

The steroid hormone receptor progesterone receptor (PR) is a ligand activated transcription factor that regulates multiple cell signaling pathways. Steroid hormone receptors such as PR and estrogen receptor (ER) have become valuable diagnostic markers for hormone-dependent diseases including breast cancer, endometriosis and potentially ovarian cancer due to their association with prognosis and therapeutic efficacy. Steroid contrast agents (CA) were studied for their ability to enhance contrast in organs that express high levels of progesterone receptors. Because the agent is lipophilic it was administered i.p. to normal CD1 mice and enhanced contrast in the uterus and ovaries. Ovarian features were evident using high-resolution ex vivo imaging on tissues resected six hours after exposure to ProGlo.

The first generation of progesterone CA, ProGlo, bound PR in vitro, and in vivo preferentially accumulated and enhanced MR signal in PR positive (but not PR negative) tumors. Building upon the success of ProGlo, the next generation of progesterone CAs was created to increase water solubility enabling multiple routes of administration. A series of CAs were synthesized with modified (1,2,3) or shortened (5,6,7) linkers between progesterone and a gadolinium chelate. Compared to ProGlo all CAs resulted in enhanced water solubility although only 5 was completely soluble in water. All CAs bound PR with 1,3,6,7 showing similar affinities as ProGlo and 5 showing 100-fold lower affinity. The compounds did not demonstrate cellular toxicity at 50 uM. To varying levels, all CAs crossed the cell membrane and activated transcription of a luciferase reporter gene fused to a PR binding element. Future studies will investigate this series of CAs for in vivo imaging of hormone receptor positive and negative tumors. These steroid hormone-conjugated CAs may help image the ovary with better spatial resolution and with deeper tissue penetration than standard transvaginal ultrasound imaging. Supported by NIH Grant R01EB014806.
The Role of The TET (Ten Eleven Translocation) Proteins in Human Uterine Leiomyoma

Antonia Navarro, Ping Yin, Diana Monsivais, Masanori Ono, Serdar E. Bulun

Department of Reproductive Biology Research, Northwestern University, Chicago, IL.

Uterine leiomyomas or fibroids are benign smooth muscle tumors of myometrial origin; despite their benign nature, they are able to undergo rapid and significant growth. They are the most common gynecological tumors in women of reproductive age, and they become symptomatic in 25–30% of all women and in up to 70% of African American women of reproductive age. The clinical symptoms associated with uterine leiomyoma are abnormal uterine bleeding, which can lead to anemia, pelvic pressure and pain; reduced fertility; and frequent pregnancy loss. Epigenetic dysregulation of individual genes has been demonstrated in leiomyoma cells; however, the in vivo genome-wide distribution of such epigenetic abnormalities remains not fully understood. We have demonstrated differences in DNA methylation in leiomyoma versus normal myometrial tissues; we observed that hypermethylation of tumor suppressor genes is a common event in leiomyoma tissues compared to normal myometrial tissues. Now, we are investigating the role of the newly identified epigenetic mark, 5-hydroxymethylcytosine (5-hmc) in human uterine leiomyoma, as well as the role of the recently discovered family of Fe (II)- and α-ketoglutarate (α-KG)-dependent dioxygenases, the TET (the ten-eleven translocation) proteins (TET1, TET2 and TET3). These enzymes are able to catalyze a three-sequential oxidation reactions: converting 5-methylcytosine (5-mC) first to 5-hydroxymethylcytosine (5-hmC), and finally 5-carboxyleycytosine (5caC). We have observed an increase in TET1 and TET3 mRNA levels in leiomyoma tissues compared to normal myometrial tissues. When we knocked down TET3 in primary human leiomyoma cells, we observed changes in PCNA and cleaved PARP. To determine a more mechanistic function, I will perform chromatin DNA immunoprecipitation (Chip) to look differential recruitment at the promoter regions of genes that I previously identified to be hypermethylated in uterine leiomyoma compared to normal myometrial tissues. Understanding the molecular mechanisms underlying the pathogenesis of uterine leiomyoma will facilitate the discovery and development of new approaches for the treatment of this disease. Supported by PO1 grant.
Colocalization of AR and ERα in human benign prostatic hyperplasia: evidence for androgen and estrogen interactions within the same cell

Tristan M. Nicholson¹,², Priyanka D. Sehgal¹, Sally A. Drew³, Wei Huang³,⁴, William A. Ricke¹,⁴,

¹Department of Urology, ²Department of Pathology and Laboratory Medicine, ³Carbone Cancer Center, University of Wisconsin, Madison, WI, USA, ⁴Department of Pathology and Laboratory Medicine, University of Rochester School of Medicine & Dentistry, Rochester, NY, USA

Benign prostatic hyperplasia (BPH) and associated lower urinary tract symptoms are common among older men. Synergy of androgens and estrogens in inducing prostate growth is well characterized in animal models of BPH. However, it is unclear which prostate cells are important for mediating androgen and estrogen synergy. Our objective was to quantitatively evaluate colocalization of androgen receptor (AR) and estrogen receptor-α (ERα) in human BPH specimens compared to normal prostate. Using a tissue microarray with normal prostate (n = 52) and BPH (n = 24), we performed multiplexed immunohistochemistry to detect AR, ERα and smooth muscle α-actin (ACTA2). Automated scanning, tissue and cell segmentation, and marker quantification were performed with a multispectral imaging platform. Consistent with previous studies, AR expression was increased in the stroma and epithelium of BPH. ERα was observed primarily in basal epithelial cells, and was increased in BPH relative to normal prostate. In the epithelium, cells expressing both AR and ERα were more prevalent in BPH compared to normal prostate. Overall, in the stroma, ERα expression and staining intensity were decreased in BPH compared to normal prostate. However, among stromal ACTA2-negative cells, there were more AR positive cells, and AR and ERα double positive cells were more prevalent in BPH compared to normal prostate (P < 0.05). These data suggest that synergy of androgens and estrogens in BPH pathogenesis may reside in AR and ERα double positive cell populations. We hypothesize that ACTA2-negative stromal cells, such as fibroblasts, are important in induction and/or maintenance of prostate growth in BPH. Taken together, these data underscore the importance of androgens and estrogens and their respective receptors in BPH. An improved understanding of hormone-responsive cell types in BPH may lead better treatment for this disease. Supported by NIH grants DK093690 and CA123199.
Regulation of Estrogen Receptor α in Prostate by Androgen Receptor Action

Hyun-Jung Park¹, Young-Chae Kim¹, and Eric C. Bolton¹

¹Department of Molecular and Integrative Physiology, School of Molecular and Cellular Biology, University of Illinois at Urbana-Champaign, Urbana, IL.

The androgen receptor (AR), a steroid hormone receptor, mediates the physiologic and pathophysiologic effects of androgens including embryonic differentiation, prostate development and prostate cancer progression. AR modulates these diverse effects by regulating transcription of AR target genes. It has been suggested that nuclear receptors including AR are able to confer multiple regulatory functions within tissues in a cell-type specific manner. However, the AR regulated genes in prostate and the mechanism(s) governing cell-type specific regulation are unknown.

Using the mouse prostate, which is comprised of multiple epithelial and mesenchymal/stromal cell types, as a model, our objective was to identify AR regulated gene products and the biologic or pathologic processes that they modulate. We have identified androgen-responsive genes in prostate stromal cells by microarray and real-time quantitative PCR analysis. Estrogen receptor alpha (ERα) mRNA and protein decreased in prostate stromal cells following androgen treatment. In addition, we observed that androgen treatment reduced ERα mRNA in urogenital sinus organ culture. These results suggest that ERα expression and, possibly, activity were down-regulated by AR action in adult prostate stromal cells and embryonic urogenital sinus mesenchyme, from which prostate stroma originates.

In the prostate, ERα is thought to play a physiologic role during development and a significant role in the pathogenesis of prostate carcinoma. However, regulatory mechanism or regulatory crosstalk between AR and ERα in prostate is undefined. We are examining the role and mechanism of AR-mediated regulation of ERα in the prostate, which will increase understanding of steroid hormone signaling in prostate development and, possibly, reveal more targeted therapies for treatment of prostate cancer.
Cellular response to antiestrogens, histone deacetylase inhibitors (HDACi) and HDACi-antiestrogen hybrid molecule in breast and ovarian cancer


Breast and ovarian are the second and fifth leading cancers causing death in women. One chemopreventive strategy for estrogen receptor (ER) positive breast cancers is treatment with antiestrogenic selective estrogen receptor modulators (SERMs). Clinical study of SERMs in ER(-) breast cancer and ovarian cancer has been very limited. Recent findings suggest that treatment of ER(-) breast cancer cells with HDAC inhibitors may sensitize the cells to the antiestrogenic effect of SERMs causing cell death. The response of an ER(-) breast cancer cell line, ovarian surface epithelial (OSE) cells, tubal epithelial cells (TEC) and ovarian cancer cells (SKOV3) was investigated, both in response to SERMs and the potentially synergistic combination with an HDAC inhibitor. An HDAC-antiestrogen hybrid drug, designed using raloxifene as a scaffold was also examined. Preliminary results from luciferase reporter and cytotoxicity assays show that the HDAC/SERM combination causes cell death in a time dependent fashion in ER(-) breast cancer cells suggesting gene reactivation and downstream gene targets as a possible mechanism of action. In contrast, while OSE, TEC and SKOV3 cells respond to estrogen and SERMs in the reporter assay, no effect on cell growth was observed at the concentration tested. These findings suggest that the combination of HDACi & SERMs have distinct effects in these cell types which may indicate separate mechanisms of action possibly due to varying ER and progesterone receptor status in these cells.
Caveolin-1, a novel mediator of classical/genomic and non-classical/rapid glucocorticoid receptor signaling in neural progenitor/stem cells

Melanie Peffer¹, Yue Zhang³, Marcia Lewis², Donald B. DeFranco¹²³

¹Program in Integrative Molecular Biology, Department of ²Pharmacology and Chemical Biology, ³Department of Neuroscience, University of Pittsburgh School of Medicine, Pittsburgh, PA 15260

While glucocorticoids (GCs) are clinically useful for a variety of conditions, their neonatal and prenatal use to aid lung maturation have been controversial due to adverse neurological and cognitive deficits that have been reported in humans and animals. Such alterations may reflect the impact of GCs on fetal neural stem/progenitor cell function. We have discovered that both genomic and rapid GC signaling in mouse neural progenitor stem cells (NPSCs) contribute to GC’s anti-proliferative effects. Furthermore, we identified caveolin-1 (Cav-1), a protein associated with lipid rafts, as a novel mediator of rapid GR actions. We are currently investigating the role of Cav-1 in modulating the anti-proliferative effects of GCs in NPSCs and determining its contribution to both genomic and rapid actions of GR. I hypothesize that Cav-1 alters GC regulation of cell cycle targets by impacting rapid and/or genomic glucocorticoid receptor (GR) signaling in NPSCs and furthermore, can impact the expression of GR target genes independent of its role in mediating GR rapid signaling. Preliminary data support the idea that anti-proliferative effects of GCs in NPSCs are influenced by Cav-1 and reveal gene-specific effects of Cav-1 on GR target genes implicated in subjects at risk for developing post-traumatic stress disorder. This work supported by T32-GM008424 and RO1 DK078394.
PTEN expression in clear cell renal cell carcinoma

S. Setty¹, S. Sonawane¹, J. Prather¹, S. Akkina², G. Chappell¹ and V. Lindgren¹

Departments of Pathology¹ and Medicine², University of Illinois Hospital and Health Science System, Chicago, IL.

The human tumor suppressor gene PTEN (phosphatase and tensin homologue deleted from chromosome 10, located on chromosome 10q231) is part of the mTOR pathway which is dysregulated in cancer. Decreased activity of PTEN, a lipid phosphatase, which functions by antagonizing PI3K/AKT/mTOR signaling, has been implicated in tumorigenesis of clear cell renal cell carcinoma (ccRCC). PTEN expression is mainly cytoplasmic. Also, nuclear PTEN protects cells from oxidative stress. The precise function of the nuclear PTEN and factors that control its localization are still unknown. We studied the relationship of PTEN protein expression with tumor nuclear grade in patients with ccRCC. We constructed a tissue microarray of 40 ccRCCs characterized using light microscopy and immunohistochemistry (IHC). We performed IHC to identify the loss of PTEN. The cores were scored for % negative staining of PTEN in the nucleus and cytoplasm. The average of multiple cores from a patient was used for analysis. The intensity of staining was also scored on a scale of 0-3, 3 being the strongest. Fuhrman nuclear grade for each tumor was determined using nuclear size, shape, number, size of nucleoli and chromatin characteristics (1-4). IHC results were analyzed in comparison to nuclear grade. Average patient age was 60 years and 37.5% of the group was female. All Fuhrman grades were represented with 7.5% of cases being grade 1, 57.5% grade 2, 2.25% grade 3 and 12.5% grade 4 tumors. Reduced PTEN IHC staining demonstrated correlation with Fuhrman grade (p=< 0.5). In summary, clear cell renal cell carcinomas have loss of PTEN protein associated with higher nuclear grade. It is well established that higher grade is associated with poorer outcome. This supports our hypothesis that loss of nuclear PTEN occurs with tumor progression.
Prostate stem/progenitor cell differentiation by retinoic acid

Guang-Bin Shi, Dan-Ping Hu, Wen-Yang Hu, Gail S. Prins

Department of Urology, University of Illinois at Chicago, Chicago, IL, 60612

Retinoic acids (RA) have been shown to have chemotherapeutic effect in prostate cancer. RA actions are mediated through RARα, β, γ and RXRα, β, γ. It has been suggested that stem cells are potential targets of cancer initiation and disease management, RA may influence the development and progression of prostate cancer by regulating stem cell differentiation and proliferation. To define the actions of RA on prostate stem cells (PSC), the present study examined RAR/RXR expression in human PSC and early stage progenitor cells (PPC) and evaluated their self-renewal and differentiation properties as a function of RA exposure.

Adult PSC/PPC were isolated from human prostates using a 3D culture system. Prostaspheres (PS) formed (<0.5%) from primary epithelial cells (PrEC) consists of self-renewing PSC and amplifying PPC. Steroid receptor expression screens revealed high levels of RARγ, RXRα, RXRβ, RARα and RARβ in PS cells making them potential RA targets. Cell division analyzed by immunodetection of CD49f in paired daughter cells demonstrated 50% of day 4 PS cells were symmetric self-renewing PSC, 18% were asymmetric dividing cells and 32% were amplifying PPC. This suggests that early stage PS cells have the abilities to both self-renew and differentiate, subsequently maintaining the stem cell pool while generating differentiated progeny. Exposure to 10 nM all trans RA markedly shifted this division pattern to 28% symmetric dividing PSC, 40% asymmetric division and 32% amplifying PPC indicating a commitment shift of PSC towards differentiation. Continued treatment of PS for 7 days with ATRA markedly augmented PSC/PPC differentiation as indicated by the formation of double layered PS structure with a significant increase in PS size. ICC and qPCR showed induction of AR, NKX3.1, CK18 and HOXB13 expression by ATRA with repression of p63 in day 7 PS. Together, the present findings document that RA is capable of driving human prostate stem cells into a differentiation pathway.

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Transgenic Expression of Cholesterol Sulfotransferase (SULT2B1b) in the Liver Prevents High-Fat Diet-Induced Obesity and Insulin Resistance in Mice.

Xiongjie Shi¹, Jiong Yan¹, Mengxi Jiang¹, Jinhan He¹, Jie Gao¹, Meishu Xu¹, Wen Xie¹.

¹Center for Pharmacogenetics and Department of Pharmaceutical Sciences, University of Pittsburgh, Pittsburgh, PA.

Cytosolic sulfotransferase (SULT) SULT2B1b are known to efficiently sulfonate cholesterol and a variety oxysterols. Although several studies have indicated that cholesterol and oxysterols play an important role in the metabolism and homeostasis of lipid and energy homeostasis, the role of SULT2B1b in the development of obesity and diabetes remains unclear. In this study, we showed that transgenic mice overexpressing SULT2B1b in the liver resistance to high-fat diet-induced obesity and improves systemic insulin sensitivity and glucose tolerance. The metabolic actions of SULT2B1b result from multiple mechanisms. SULT2b1b-tg mice had increased whole body energy expenditure and fatty acid oxidation, decreased lipid accumulation, bile acid level and cholesterol level in liver. Gene and protein expression analysis showed that the levels of several critical lipogenic and gluconeogenic genes were significantly decreased in HFD-fed SULT2b1b-tg mice. In vivo and in vitro results showed that overexpression of SULT2B1b inhibited liver X receptors (LXR) pathway which is vital in lipid homeostasis. Overexpression of SULT2B1b also suppressed the acetylation of hepatocyte nuclear factor 4 (HNF4), resulted in impaired nuclear translocation of HNF4 and subsequently the down-regulation of G6P and PEPCK, which are two important enzyme in gluconeogenesis. These findings reveal an important role of SULT2B1b in lipid, glucose and energy homeostasis and point to SULT2B1b as potential therapeutic targets for metabolic disorders. Supported by
Poster #36

**RHOX homeobox regulation of Ins2 in granulosa cells of the ovary**

Cassandra Showmaker, Raquel Brown, Kanako Hayashi, and James A. MacLean II.

*Department of Physiology, Southern Illinois University, School of Medicine, Carbondale, IL 62901*

Homeobox genes are well-established transcriptional regulators of embryonic development, but their downstream targets have been relatively elusive. Here we characterize *Ins2* as a downstream target of RHOX homeobox factors. While INS2 is classically produced by islet cells in the pancreas, we found that the *Ins2* gene is expressed locally in testis and ovary. In the testes, *Ins2* requires RHOX5 to be expressed. However, in the ovary, *Ins2* remains highly expressed in granulosa cells of *Rhox5*-null mice. RHOX5 induces *Rhox8* expression in pre-antral granulosa cells and is relatively silent in peri-ovulatory follicles. However, *Rhox8* does not peak until after the ovulatory LH surge when *Ins2* reaches its maximal expression level. To determine whether RHOX8 regulates *Ins2*, we characterized the *Ins2* promoter and discovered a critical homeobox binding element that was required for maximal transcription in granulosa cells. We propose that the induction of *Rhox8* by progesterone, after the normal window of RHOX5 has passed, may explain why *Rhox5*-null female mice display apparently normal fertility, as RHOX8 may be capable of redundant stimulation of target genes that are essential for ovulation. Many RHOX-regulated genes identified to date encode proteins regulating metabolism. Thus, study of the *Rhox* cluster may provide a useful model in elucidating the mechanism by which diseases such as diabetes and metabolic syndrome result in reduced fertility. Supported by NIH/NICHD 55268 and the SIUC Research Rookies Program.
Ecdysone receptor (EcR) binding sites through Drosophila melanogaster development

R.F. Spokony¹, J.N. Cohen¹, C.A. Morrison¹, N. Bild¹, N. Negre¹, K.P. White¹

¹Institute for Genomics & Systems Biology, The University of Chicago, Chicago, IL.

The steroid hormone ecdysone coordinates development. Ecdysone action is mediated through a ligand activated nuclear receptor, the ecdysone receptor (EcR) and the cofactor ultraspiracle. EcR mutants display developmental defects in many ecdysone regulated processes. To characterize direct targets of EcR through development, we identified EcR binding sites in whole animals using whole genome chromatin immunoprecipitation assays with sequencing (ChIP-seq) at 8 timepoints from the beginning of metamorphosis through adulthood. EcR binds an average of 2345 genomic locations which map to an average of 1612 genes. Up to 45% of the genes bound at one timepoint are known to be differentially expressed upon ecdysone treatment or loss of EcR function. EcR binding is highly dynamic throughout development, on average, 20% of genes were unique to one timepoint examined. A small number of genes (~100) was bound at all timepoints examined including primary ecdysone targets, ecdysone producing enzymes, and nuclear receptors (Hnf4, Hr39, Hr4 and Hr46). Other important ecdysone response genes are bound more dynamically across the timepoints. Interestingly, ftz-f1 (a midprepupal competence factor and nuclear receptor) and Cyp18a1 (an ecdysone degrading enzyme) are not bound by EcR until midprepupal development, when their expression is increasing and the ecdysone titer is decreasing. Analysis of target gene GO categories revealed that EcR target genes at all timepoints were enriched for ecdysone regulated processes including endocytosis, circadian rhythm, Wnt signaling and glycolysis, at almost every timepoint. We found new targets of EcR in known and new pathways. Interestingly, EcR binds near 15 genes involved in the insulin response across development, half of which require EcR at midprepupal development. These results suggest feedback from the ecdysone pathway to the insulin and circadian rhythm pathways that control ecdysone synthesis.
Transgenic approach reveals strong protective effects of vitamin D receptor signaling on podocytes

Youli Wang, Dilip K. Deb, Tao Sun, Zhongyi Zhang, and Yan Chun Li

Department of Medicine, The University of Chicago, Chicago, IL

Background: Podocytes play a key role in maintaining the integrity of the glomerular filtration barrier, and podocyte injury is a major cause for renal dysfunction in diabetic nephropathy. Clinical and animal studies have demonstrated potent anti-proteinuric activity for vitamin D and its analogs, suggesting podocytes as important reno-protective target of vitamin D signaling.

Methods: To test this hypothesis, we targeted Flag-tagged human (h) VDR to podocytes in DBA/2J mice using the 2.5 kb podocin gene promoter. The transgenic (Tg) mice were analyzed using the model of streptozotocin (STZ)-induced diabetic nephropathy.

Results: Podocyte-specific expression of hVDR was confirmed by Western blotting and immunostaining in hVDR-Tg mice. Diabetic hVDR-Tg mice exhibited less albuminuria compared to wild-type (WT) counterparts. While treatment with a low dose vitamin D analog doxercalciferol (Dox, 30 ng/kg bw, i.p. 3x/wk) had little effects on the progression of diabetic nephropathy in WT mice, this treatment almost completely prevented albuminuria and markedly reduced glomerular fibrosis in diabetic hVDR-Tg mice. WT1 and synaptopodin staining demonstrated decreased podocyte injury, and TUNEL assays showed attenuation of podocyte apoptosis in the hVDR-Tg mice. Dox treatment also prevented the elevation of renal renin and fibronectin and preserved the expression of nephrin in the hVDR-Tg mice. Moreover, when the hVDR transgene was bred into VDR-null mice to generated VDR knock-out (KO) mice that express hVDR only in podocytes, the hVDR transgene was able to partially rescue the severe renal damages seen in VDR KO mice in the STZ-diabetic model, manifested by marked reduction in albuminuria and podocyte loss. In podocyte culture exposure to high glucose (30 mM) induced apoptosis with up-regulation of p-Erk, p38, Bad and Bak, and vitamin D treatment blocked this pro-apoptotic pathway and podocyte apoptosis.

Conclusion: Taken together, these data provide strong evidence that vitamin D-VDR signaling plays a critical role in the protection of podocytes from diabetic injury.
CCN3/NOV Gene Expression in Human Prostate Cancer is Directly Suppressed by the Androgen Receptor

Longtao Wu¹, Christine Runkle¹, Hong-Jian Jin¹, Jianjun Yu¹, James Li¹, Ximing Yang², Timothy Kuzel¹,², Chung Lee², Jindan Yu¹,²

¹Division of Hematology/Oncology, Department of Medicine, ²Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL 60611.

Abstract

Androgen receptor (AR) plays essential roles during prostate cancer progression. With genome-wide AR binding sites mapped to high resolution, studies have recently reported AR as a transcriptional repressor. How AR inhibits gene expression and how this contributes to prostate cancer, however, are incompletely understood. Through meta-analysis of microarray data, here we nominate NOV as a top androgen-repressed gene. We show that NOV is directly suppressed by androgen through the AR. AR occupies the NOV enhancer and communicates with the NOV promoter through DNA looping. AR activation recruits the polycomb group protein EZH2, which subsequently catalyzes H3K27 tri-methylation around the NOV promoter, thus leading to repressive chromatin remodeling and epigenetic silencing. Concordantly, AR and EZH2 inhibition synergistically restored NOV expression. NOV is down-regulated in human prostate cancer wherein AR and EZH2 are up-regulated. Functionally, NOV inhibits prostate cancer cell growth in vitro and in vivo. NOV reconstitution reverses androgen-induced cell growth and NOV knockdown drives androgen-independent cell growth. In addition, NOV expression is restored by hormone-deprivation therapies in mice and prostate cancer patients. Therefore, using NOV as a model gene we gained further understanding of the mechanisms underlying AR-mediated transcriptional repression. Our findings establish a tumor suppressive role of NOV in prostate cancer and suggest that one important, but previously underestimated, manner by which AR contributes to prostate cancer progression is through inhibition of key tumor suppressor genes.

Keywords: Androgen receptor, Polycomb EZH2, NOV/CCN3
Alternative Splicing of CARM1 in Breast Cancer

Lu Wang, Wei Xu

McArdle Laboratory for Cancer Research,
University of Wisconsin-Madison, Madison, WI 53706

Estrogen receptor alpha (ERα) is expressed in 70% of breast cancer and is the main target for endocrine therapies. ERα regulates gene expression through recruitment of multiple cofactors including a histone arginine methyltransferase CARM1. The functions of CARM1 protein in breast cancer are controversial; some studies implicate CARM1 in promotion of cancer cell growth while others suggest CARM1 makes tumor cells more differentiated. To further investigate the discrepancy, we cloned human cDNA of CARM1 mRNA from MCF7 breast cancer cells and found that exon 15 was deleted from the predicted full-length CARM1 cDNA sequence, although the genomic DNA is intact. In fact, CARM1 exon15 deleted form (CARM1ΔE15) was the predominant form of CARM1 mRNA existing in a variety of human cancer cell lines and primary breast tumors. The process of alternative splicing (AS) is widely deregulated in cancer; however, the cause-effect relationship between splicing changes of a gene and cancer initiation and progression often remains unclear. Our data suggest that CARM1 is alternatively spliced in cancer. We found that CARM1ΔE15 protein is more sensitive to Hsp90 inhibitors for degradation than full-length CARM1, implying that CARM1ΔE15 is an oncogenic client of Hsp90 in breast cancer. Furthermore, CARM1ΔE15 promotes cell transformation, suggesting it to be an oncogenic form of CARM1 created by cancer-associated AS of CARM1. In contrast, CARM1 full length (FL) protein inhibits ID proteins, which are promoters of breast cancer lung metastasis. Our findings suggest that two splice variants of CARM1 have opposing functions in carcinogenesis: CARM1FL has tumor suppressor function while CARM1ΔE15 is oncogenic. Future studies will examine the regulatory mechanism of CARM1 alternative splicing in breast cancer and test the possibility of targeting CARM1ΔE15 for breast cancer treatment. This work is supported by National Institutes of Health Grant CA125387 (to W.X.), a Department of Defense Era of Hope Scholar Award, Mirus Research Award, Shaw Scientist Award from the Greater Milwaukee Foundation (to W.X.).
Role of ER-Coactivator SRC-1 in Breast Cancer Metastasis to the Bone

Watters, RJ\textsuperscript{1}, Hartmaier, RJ\textsuperscript{1}, Rios, J\textsuperscript{2}, Lee, A\textsuperscript{1}, Anderson, C\textsuperscript{3}, Brufsky, A\textsuperscript{2}, Oesterreich, S\textsuperscript{1}

1. University of Pittsburgh, Department of Pharmacology and Chemical Biology, UPCI, WCRC, Pittsburgh, PA, 2. Department of Hematology/Oncology, University of Pittsburgh, Pittsburgh, PA, 3. Department of Radiology, University of Pittsburgh, Pittsburgh, PA

Bone is one of the major sites of distant metastases for breast cancer and occurs in ~65-80% of patients. Bone metastases driven by osteoclast resorption result in serious bone fractures, pain, hypercalcemia, and nerve compression. According to the “seed and soil” hypothesis, the bone microenvironment provides a rich milieu for growth due to its mineralized extracellular matrix containing abundant growth factors and ionized calcium for cancer cells. Higher rates of bone recurrence are more frequently observed in individuals with ER-positive luminal breast cancer and our clinical data demonstrates that more than 50% of ER-positive tumors develop bone metastases. ER co-regulators are proteins that bind to and modulate the activity of ER and we have demonstrated that SRC-1 is a critical regulator of estrogen response in bone mineral density. Understanding SRC-1 action in ER response in bone is significant as bone is a major site of breast cancer metastasis, thus raising the possibility that SRC-1 may be involved in forming the metastatic niche for breast cancer metastasis to bone. Migration assays have revealed that the presence of SRC-1 is necessary for osteoclast migration. However, co-cultures of T\textsubscript{47D} with RAW264.7 cells indicate that differentiation of osteoclasts does not depend on SRC-1. In summary, we demonstrate that SRC-1 and ER may be interacting together to facilitate breast cancer metastasis to bone. Further testing of this hypothesis is being conducted in mouse and rat metastasis models and patient samples.
VS-110: A novel vitamin D receptor modulator with cardiovascular protective effects in 5/6 nephrectomized uremic rats

J. Ruth Wu-Wong, Megumi Kawai, Yung-wu Chen, Masaki Nakane

Vidasym, Chicago, IL

Vitamin D receptor modulators (VDRMs) such as calcitriol, paricalcitol and doxercalciferol are commonly used to manage hyperparathyroidism secondary to chronic kidney disease (CKD). A majority of CKD patients die from cardiovascular complications. Clinical observations demonstrate that VDRM therapy may provide cardiovascular and survival benefit for CKD patients. However, current on-market VDRMs have a narrow therapeutic index (TI) at 1-4-fold (estimated from the hypercalcemic toxicity and PTH suppressing efficacy). Hypercalcemia remains a serious concern, which leads to the need for frequent drug dose titration and serum calcium monitoring. Significant clinical benefit can be derived from a VDRM with expanded TI and cardiovascular protective effects. The 5/6 nephrectomized (NX) male Sprague–Dawley rats at Week 6 after the surgery exhibited established uremia, elevated parathyroid hormone (PTH), endothelial dysfunction and left ventricular hypertrophy. Treatment of 5/6 NX rats by VS-110, a novel VDRM, at 0.01 – 1.0 μg/kg (oral gavage, once daily, for two weeks) suppressed serum PTH effectively without raising serum calcium, demonstrating a >50-fold TI. Similar results were obtained when VS-110 was given to 5/6 NX rats by i.p., 3x/week for two weeks. When the 5/6 NX uremic rats were treated with VS-110 (0.01 - 1 μg/kg) for two weeks, VS-110 improved endothelium-dependent aortic relaxation, reduced left ventricular (LV) fibrosis and attenuated LV hypertrophy in a dose-dependent manner without affecting serum calcium. Real-Time PCR showed that VS-110 induced CY-P24A1 and CD14 expression in HL-60 cells with EC$_{50}$ values at 6.8 and 0.4 nM, respectively. VS-110 induced HL-60 differentiation with an EC$_{50}$ value at 1.7 nM (vs. calcitriol at 13.9 nM) and inhibited the proliferation of primary human keratinocytes with an IC$_{50}$ value at 1.4 nM (vs. calcitriol at 10 nM). These studies demonstrate that VS-110 is a novel VDRM with greatly expanded TI and an overall therapeutic product profile that supports clinical development for expanded use in pre-dialysis CKD patients to realize the cardiovascular protective effects of VDR activation.
Histone H3R17me2a Mark Recruits Human RNA Polymerase-Associated Factor 1 Complex to Activate Transcription

Wei Xu and Jiacai Wu

McArdle Laboratory for Cancer Research, University of Wisconsin, 1400 University Avenue, Madison, WI 53706, USA.

The histone arginine methyltransferase CARM1 is a coactivator for a number of transcription factors including nuclear receptors. Although CARM1 and its asymmetrically deposited dimethylation at histone H3 arginine 17 (H3R17me2a) are associated with transcription activation, the mechanism by which CARM1 activates transcription remains unclear. Using an unbiased biochemical approach, we discovered that the transcription elongation-associated PAF1 complex (PAF1c) directly interacts with H3R17me2a. PAF1c binds to histone H3 tails harboring dimethylation at R17 in CARM1-methylated histone octamers. Knockdown of either PAF1c subunits or CARM1 affected transcription of CARM1-regulated, estrogen responsive genes. Furthermore, either CARM1 knockdown or CARM1 enzyme deficient mutant knockin resulted in decreased H3R17me2a accompanied by the reduction of PAF1c occupancy at the proximal promoter estrogen responsive elements (EREs). In contrast, PAF1c knockdown elicited no effects on H3R17me2a but reduced H3K4me3 level at EREs. These observations suggest that, apart from PAF1c’s established roles in directing histone modifications, PAF1c acts as an arginine methyl histone effector that is recruited to promoters and activates a subset of genes including targets of estrogen signaling. This work is supported by National Institutes of Health Grant CA125387 (to W.X.), a Department of Defense Era of Hope Scholar Award, Mirus Research Award, Shaw Scientist Award from the Greater Milwaukee Foundation (to W.X.).
Expression Profiling of Nuclear Receptors and Characterizing the Essential Roles of NR4A Subfamily in Human Uterine Leiomyoma.

Hanwei Yin, Jay H. Lo, Ji-Young Kim, and Debra Rata Chakravarti

Division of Reproductive Biology Research, Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University, Chicago, IL.

Uterine leiomyomas (UL) are benign, fibrotic smooth muscle tumors. While the GnRH analog leuprolide acetate that suppresses gonadal steroid hormones is used as a treatment, it has significant side effects thereby limiting its use. Availability of more effective therapy is limited because of a lack of understanding of molecular underpinnings of the disease. While ovarian steroid hormones estrogen and progesterone and their receptors are clearly involved, the role of other nuclear receptors in UL is not well defined. We used quantitative real-time PCR to systematically profile the expression of 48 nuclear receptors (NRs) and identified several NRs that were aberrantly expressed in UL. Among others, expressions of NR4A subfamily including NGFIB (NR4A1), NURR1 (NR4A2), and NOR1 (NR4A3) were dramatically suppressed in UL compared with the matched myometrium. Restoration of expression of each of these NR4A members in the primary leiomyoma smooth muscle cells (LSMCs) decreased cell proliferation. Importantly, NR4As regulate expressions of the pro-fibrotic stimuli including transforming growth factor β3 (TGFβ3) and SMAD3, and several collagens which are key components of the extracellular matrix (ECM). Finally, we identify NR4A members as targets of leuprolide acetate treatment. Together, our results implicate several NRs including the NR4A subfamily in UL etiology and identify NR4As as potential therapeutic targets of UL. This is the abstract supported in part by NIH Grant PO1 HD57877.
FoxA1 as a double-edged sword in prostate cancer progression

Hong-Jian Jin¹, Jonathan C. Zhao¹, Irene Ogden¹, Raymond Bergan¹,², Jindan Yu¹,²

¹Division of Hematology/Oncology, Department of Medicine, ²Robert H. Lurie Comprehensive Cancer Center, Northwestern University Feinberg School of Medicine, Chicago, IL 60611.

FoxA1 is a pioneering transcription factor of the androgen receptor (AR), indispensable for the lineage-specific gene expression in the prostate. With recent discoveries of recurrent FoxA1 mutations in human prostate tumors, the important function of FoxA1 has just begun to be investigated. Here, through genomic analysis we reveal that FoxA1 regulates two distinct oncogenic processes via disparate mechanisms. FoxA1 induces cell growth requiring the AR pathway. On the other hand, FoxA1 inhibits cell motility and epithelial-to-mesenchymal transition (EMT) through AR-independent and -opposing mechanisms. Using orthotopic mouse models we further show that FoxA1 inhibits prostate tumor metastasis in vivo. Concordant with these double-edged sword effects, FoxA1 expression is slightly up-regulated in localized prostate cancer wherein cell proliferation is the main feature, but is remarkably down-regulated when the disease progresses to metastatic stage for which cell migration and EMT would have been essential. Importantly, recently identified mutations of FoxA1 drastically attenuated its ability to suppress cell invasion. Taken together, our findings illustrate an AR-independent function of FoxA1 as a metastasis inhibitor and provide a first mechanism by which recurrent FoxA1 mutations contribute to prostate cancer progression.
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<tr>
<td>Michael Akroush</td>
<td>DePaul University</td>
<td><a href="mailto:mikeakroush@aol.com">mikeakroush@aol.com</a></td>
</tr>
<tr>
<td>Lydia Arbogast</td>
<td>SIU</td>
<td><a href="mailto:lARBOGAST@SIUMED.EDU">lARBOGAST@SIUMED.EDU</a></td>
</tr>
<tr>
<td>Kannan Athilakshmi</td>
<td>University of Illinois at Urbana-Champaign</td>
<td><a href="mailto:Kalki32@illinois.edu">Kalki32@illinois.edu</a></td>
</tr>
<tr>
<td>Lynn Birch</td>
<td>University of Illinois at Chicago</td>
<td><a href="mailto:Lbirch@uic.edu">Lbirch@uic.edu</a></td>
</tr>
<tr>
<td>Eric Bolton</td>
<td>University of Illinois at Urbana-Champaign</td>
<td><a href="mailto:BoltonEC@Life.Illinois.EDU">BoltonEC@Life.Illinois.EDU</a></td>
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<tr>
<td>Esther Calderon</td>
<td></td>
<td><a href="mailto:Ecalde4@uic.edu">Ecalde4@uic.edu</a></td>
</tr>
<tr>
<td>Abha Chalpe</td>
<td>Northwestern University</td>
<td><a href="mailto:Abha.Chalpe@Northwestern.edu">Abha.Chalpe@Northwestern.edu</a></td>
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<tr>
<td>Congcong Chen</td>
<td>University of Illinois at Urbana-Champaign</td>
<td><a href="mailto:Archmage.3C@Gmail.com">Archmage.3C@Gmail.com</a></td>
</tr>
<tr>
<td>Mathew Cherian</td>
<td>University of Illinois at Urbana-Champaign</td>
<td><a href="mailto:Mcherian@illinois.edu">Mcherian@illinois.edu</a></td>
</tr>
<tr>
<td>Marilia Cordeiro</td>
<td>Northwestern University</td>
<td><a href="mailto:MariliaCordeiro@Gmail.com">MariliaCordeiro@Gmail.com</a></td>
</tr>
<tr>
<td>Juanmahel Davila</td>
<td>University of Illinois at Champaign-Urbana</td>
<td><a href="mailto:Davilo@illinois.edu">Davilo@illinois.edu</a></td>
</tr>
<tr>
<td>Donald DeFranco</td>
<td>University of Pittsburgh</td>
<td><a href="mailto:Dod1@Pitt.edu">Dod1@Pitt.edu</a></td>
</tr>
<tr>
<td>Anushka Dikshit</td>
<td>University of Illinois at Urbana-Champaign</td>
<td><a href="mailto:Anushdd@Gmail.com">Anushdd@Gmail.com</a></td>
</tr>
<tr>
<td>Matthew Dyson</td>
<td>Northwestern University</td>
<td><a href="mailto:M-Dyson@Northwestern.edu">M-Dyson@Northwestern.edu</a></td>
</tr>
<tr>
<td>Erfan Eilati</td>
<td>Southern Illinois University</td>
<td><a href="mailto:Erfan.Eilati@Gmail.com">Erfan.Eilati@Gmail.com</a></td>
</tr>
<tr>
<td>Lamiaa El-Shennawy</td>
<td>University of Illinois at Chicago</td>
<td><a href="mailto:Lelshe2@Uic.edu">Lelshe2@Uic.edu</a></td>
</tr>
<tr>
<td>Michael Fritsch</td>
<td>Northwestern University</td>
<td><a href="mailto:Mfritsch@LurieChildrens.org">Mfritsch@LurieChildrens.org</a></td>
</tr>
<tr>
<td>Georgia Gaconnet</td>
<td>Northwestern University</td>
<td><a href="mailto:G-Gaconnet@Northwestern.edu">G-Gaconnet@Northwestern.edu</a></td>
</tr>
<tr>
<td>Geoffrey Greene</td>
<td>University of Chicago</td>
<td><a href="mailto:Ggreene@Uchicago.edu">Ggreene@Uchicago.edu</a></td>
</tr>
<tr>
<td>Atieh Hajirahimkhan</td>
<td>University of Illinois at Chicago</td>
<td><a href="mailto:Ahajir2@Uic.edu">Ahajir2@Uic.edu</a></td>
</tr>
<tr>
<td>Buck Hales</td>
<td>SIU School of Medicine</td>
<td><a href="mailto:Dhales@Siumed.edu">Dhales@Siumed.edu</a></td>
</tr>
<tr>
<td>Terry Horton</td>
<td>Northwestern University</td>
<td><a href="mailto:Thorton@Northwestern.edu">Thorton@Northwestern.edu</a></td>
</tr>
<tr>
<td>Teresa Horton</td>
<td></td>
<td><a href="mailto:Thorton@Northwestern.edu">Thorton@Northwestern.edu</a></td>
</tr>
</tbody>
</table>
Dan-ping (Grace) Hu  
University of Illinois at Chicago  
dhu@uic.edu

Wen-Yang Hu  
wyhu@uic.edu

Emily Isgur  
SIU School of Medicine  
eisgur@siu.edu

Young Jeong  
University of Illinois at Chicago  
yjeong@uic.edu

Mengxi Jiang  
University of Illinois at Urbana-Champaign  
mej39@pitt.edu

Joan Jorgensen  
University of Wisconsin  
jsjorgensen@wisc.edu

Shruti Kamath  
DePaul University  
shruti_kamath_in@hotmail.com

Irida Kastrati  
University of Illinois at Chicago  
ikastr2@uic.edu

Young-Chae Kim  
University of Illinois at Champaign-Urbana  
ychaekim@uiuc.edu

So-youn Kim  
Northwestern University  
so-youn-kim@northwestern.edu

Julie Kim  
Northwestern University  
j-kim4@northwestern.edu

Jingjing Kipp  
DePaul University  
jkipp@depaul.edu

CheMyong Ko  
University of Illinois  
jayko@illinois.edu

Kwi Hye Koh  
khkoh@uic.edu

Kenneth Korach  
NIH

Nancy Krett  
Northwestern University  
n-krett@northwestern.edu

Takeshi Kurita  
Northwestern University  
t-kurita@northwestern.edu

Nawneet Kurrey  
SIU Carbondale  
nawneetkurrey@gmail.com

Monica Laronda  
Northwestern University  
m-laronda@northwestern.edu

Irene Lee  
Northwestern University  
irenelee2014@u.northwestern.edu

Jin Kyung Lee  
jinkyung@uic.edu

Samantha Lewis  
University of Wisconsin-Madison  
srlewis2@wisc.edu

Quanxi Li  
University of Illinois at Urbana-Champaign  
quanxili@illinois.edu

Ikenna Madueke  
University of Illinois at Chicago  
imadue2@uic.edu

Kelly Mayo  
Northwestern University  
k-mayo@northwestern.edu

Kristen Meldi  
Northwestern University  
kristenmeldi2010@u.northwestern.edu
GLNRC participants

Geonyoung Ahn
University of Wisconsin-Madison
gahn2@wisc.edu

Elaine Alarid
University of Wisconsin-Madison
alarid@oncology.wisc.edu

Courtney Andersen
University of Pittsburgh
ryann@upmc.edu

Amir Bahreini
University of Pittsburgh School of Medicine
ryann@upmc.edu

Gleb Baida
Northwestern University
g-baida@northwestern.edu

Grant Barish
Northwestern University
grant.barish@northwestern.edu

Ryan Bourgo
University of Chicago
bourgorj@uchicago.edu

Ashley Brinkman
University of Wisconsin-Madison
abrinkman@wisc.edu

Eric Carter
University of Michigan Medical School
ericlc@umich.edu

Debu Chakravarti
Northwestern University
debu@northwestern.edu

Carlos Coriano
University of Wisconsin-Madison
coriano@wisc.edu

Dilip K Deb
The University of Chicago
ddeb@bsd.uchicago.edu

Ruby Dhar
The University of Chicago
rdhar@uchicago.edu

Xin Duan
University of Cincinnati
xin.duan@uc.edu

Sean Fanning
hosfield@uchicago.edu

Maxfield Flynn
Northwestern University
mflynn005@northwestern.edu

Jonna Frasor
University of Illinois at Chicago
jfrasor@uic.edu

Amanda Gardner
University of Cincinnati
amanda.gardner@uc.edu

Chun Guo
University of Cincinnati
chun.guo@uc.edu

Nirupama Gupta
University of Michigan Medical School
nirupama@umich.edu

Grace Guzman
graceguz@uic.edu

Stephen Hammes
University of Rochester
stephen_hammes@urmc.rochester.edu

Alison Hantak
University of Illinois at Urbana-Champaign
hantak@illinois.edu

David Hosfield
University of Chicago
sfanning@uchicago.edu

Jordy Hsiao
University of Iowa
jordy-hsiao@uiowa.edu
Hanwei Yin
hanweiyin2015@u.northwestern.edu

Ping Yin
p-yin@northwestern.edu

Hao Zeng
University of Wisconsin-Madison
zeng8@wisc.edu

Jinsong Zhang
University of Cincinnati
jinsong.zhang@uc.edu

Hong Zhao
h-zhao@northwestern.edu

Zibo Zhao
University of Wisconsin-Madison
zzhao32@wisc.edu

Abena Agyeman
The University of Chicago
aagyeman@medicine.bsd.uchicago.edu

Anne Keener
akl21@pitt.edu

Melanie Peffer
University of Toledo
mew43@pitt.edu

Ruth Wu-Wong
ruth.wuwong@prodigy.net