Abstracts of papers presented
at the 2010 meeting on

NUCLEAR RECEPTORS & DISEASE

August 31–September 4, 2010

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ENVIRONMENT
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CANCER
Nuclear Receptors
NR
METABOLIC DISEASE
THERAPY

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

Abstracts of papers presented
at the 2010 meeting on

NUCLEAR RECEPTORS & DISEASE

August 31–September 4, 2010

Arranged by

Ron Evans, *HHMI/Salk Institute for Biological Studies*
Jerrold Olefsky, *University of California, San Diego*
Keith Yamamoto, *University of California, San Francisco*

Cold Spring Harbor Laboratory
Cold Spring Harbor, New York

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Cover: The illustration shows Nuclear Receptors and their control of diverse physiologic pathways (provided by Ronald Evans).

The relationship between receptor's function and physiology illustrates how receptors are both controlled by environmental, physiologic and pathologic changes as well as illustrating the potential of receptors to impact on both the progression and treatment of disease.

NUCLEAR RECEPTORS & DISEASE

Tuesday, August 31 – Saturday, September 4, 2010

Tuesday	7:30 pm	**1** Keynote Speakers
Wednesday	9:00 am	**2** Physiology and Metabolism
Wednesday	2:00 pm	**3** Poster Session I
Wednesday	4:30 pm	Wine and Cheese Party *
Wednesday	7:30 pm	**4** Inflammation and Metabolic Disease
Thursday	9:00 am	**5** Ligands and Cofactors
Thursday	2:00 pm	**6** Poster Session II
Thursday	7:30 pm	**7** Cancer
Friday	9:00 am	**8** Mechanisms of Insulin Resistance and Adipogenesis
Friday	2:00 pm	**9** Inflammation II
Friday	6:00 pm	Concert
	7:00 pm	Banquet
Saturday	9:00 am	**10** Chromatin and Transcription

Poster sessions are located in *Bush Lecture Hall*

* *Airslie Lawn*, weather permitting

Mealtimes at Blackford Hall are as follows:

Breakfast 7:30 am-9:00 am

Lunch 11:30 am-1:30 pm

Dinner 5:30 pm-7:00 pm

Bar is open from 5:00 pm until late

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PROGRAM

TUESDAY, August 31—7:30 PM

SESSION 1 KEYNOTE SPEAKERS

Chairperson: **K. Yamamoto,** University of California, San Francisco

Nuclear receptor modulators (NuRMs) in worms
Steven A. Kliewer, David J. Mangelsdorf.
Presenter affiliation: University of Texas Southwestern Medical School, Dallas, Texas. 1

PPARγ and the anti-diabetic PPARγ ligands—A new look at an old friend
Bruce Spiegelman.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts. 2

WEDNESDAY, September 1—9:00 AM

SESSION 2 PHYSIOLOGY AND METABOLISM

Chairperson: **A. Saltiel,** University of Michigan, Ann Arbor

Integrating metabolic control by NAD+ sensors
Johan Auwerx.
Presenter affiliation: Ecole Polytechnique Federale de Lausanne (EPFL), Lausanne, Switzerland. 3

The nuclear bile acid receptor FXR and SIRT1 deacetylase form a dynamic interactive regulatory network controlling hepatic lipid metabolism
Jongsook K. Kemper.
Presenter affiliation: University of Illinois at Urbana-Champaign, Urbana, Illinois. 4

NHR-49 as chief economist
Mark Van Gilst.
Presenter affiliation: Fred Hutchinson Cancer Research Center, Seattle, Washington. 5

Gene/environment influence on skeletal muscle insulin sensitivity in type 2 diabetic patients.
Juleen Zierath.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 6

PPARα orchestrates sexual dimorphism in hepatic functions
Walter Wahli, Gianpaolo Rando, Nicolas Leuenberger.
Presenter affiliation: University of Lausanne, Lausanne, Switzerland. 7

Oxysterols are ligands for the orphan nuclear receptor RORγt, a key regulator of Th17 cell development
Katherine Rouleau, Xiaoshan Min, Zhulun Wang, Anke Konrad, John E. Sims, Antony Symons.
Presenter affiliation: Amgen Inc., Seattle, Washington. 8

Circadian transcriptional repressors Cry1 and Cry2 mediate transrepression by the glucocorticoid receptor
Katja A. Lamia, Grant D. Barish, Ruth T. Yu, N Henriette Uhlenhaut, Johan W. Jonker, Jamie Whyte, Michael Downes, Ronald M. Evans.
Presenter affiliation: The Salk Institute for Biological Studies, La Jolla, California; The Scripps Research Institute, La Jolla, California. 9

WEDNESDAY, September 1—2:00 PM

SESSION 3 POSTER SESSION I

Promoter elements and protein function of the atypical corepressor TNIP1 indicate an expression feedback loop
Igor Gurevich, Carmen Zhang, Priscilla Encarnacao, Vincent Ramirez, Brian Aneskievich.
Presenter affiliation: University of Connecticut, Storrs, Connecticut. 10

Image-based analysis of ligand-specific coregulator recruitment to an estrogen receptor α-occupied promoter—An application for high content screening
Felicity J. Ashcroft, Michael Bolt, Michael A. Mancini.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 11

Conditional knockdown of p70S6 kinase in liver protects against hepatic steatosis and systemic insulin resistance
Eun Ju Bae, Jianfeng Xu, Jin-Long Chen, Jerrold M. Olefsky.
Presenter affiliation: UCSD, La Jolla, California. 12

Loss of ER-regulated microRNAs is fundamental to hormone-independent breast cancer cells
Shannon T. Bailey, Housheng He, Myles Brown.
Presenter affiliation: Dana-Farber Cancer Institute/Harvard Medical School, Boston, Massachusetts. 13

Effects of the selective glucocorticoid receptor modulator Compound A on Hsp70's expression and functionality in the combat of inflammation
Ilse M. Beck, Ruben Hoya Arias, Wim Vanden Berghe, Ali Adem Bahar, Guy Haegeman, Karolien De Bosscher.
Presenter affiliation: University of Ghent, Gent, Belgium. 14

Structure elucidation of endogenous ligands of nuclear hormone receptors and other signaling molecules through Differential Analysis by 2D NMR Spectroscopy
Parag Mahanti, Neelanjan Bose, Axel Bethke, Joshua C. Judkins, Joshua Wollam, Adam Antebi, Frank C. Schroeder.
Presenter affiliation: Cornell University, Ithaca, New York. 15

The immunomodulatory glycan LNFPIII attenuates metabolic syndrome in HFD-induced obesity
Prerna Bhargava, Changlin Li, Sihao Liu, Kristopher J. Stanya, Donald A. Harn, Chih-Hao Lee.
Presenter affiliation: Harvard School of Public Health, Boston, Massachusetts. 16

Structural studies of transcriptional nuclear receptor complexes involving ERR and PGC-1α
Isabelle M L. Billas, Maria Takács, Maxim Petoukhov, Borries Demeler, Javier Perez, Dmitri I. Svergun, Dino Moras.
Presenter affiliation: IGBMC (Institut de Génétique et de Biologie Moléculaire et Cellulaire), Illkirch, France. 17

***In vitro* biotools for NR research—From whole cell biosensors and –omics based methods to use of precision cut organ slices and ussing chambers**
Toine F. Bovee, Peter J. Hendriksen, Jeroen C. Rijk, Ad A. Peijnenburg, Ron L. Hoogenboom, Michel W. Nielen.
Presenter affiliation: RIKILT-Institute of Food Safety, WUR, Wageningen, the Netherlands. 18

Glucocorticoids enhance the expression and function of the NLRP3 inflammasome
John M. Busillo, John A. Cidlowski.
Presenter affiliation: Molecular Endocrinology Group, Laboratory of Signal Transduction, Research Triangle Park, North Carolina. 19

Loss of IKKξ improves energy expenditure, diet-induced inflammation and hepatic steatosis independently of body weight
Shian-Huey Chiang, Jonathan Hung, Lynn Geletka, Xiaoling Peng, Nathan Qi, Alan Saltiel.
Presenter affiliation: University of Michigan, Ann Arbor, Michigan. 20

Regulation of the histone demethylase LSD1 by nuclear hormone receptors.
Erin A. Clark, Yujiang Shi.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 21

Progesterone receptor activity maintains uterine quiescence through direct regulation of caspase-3 action in the pregnant uterus.
Pancharatnam Jeyasuria, Kalpana Subedi, Arvind Suresh, Jennifer Condon.
Presenter affiliation: Magee Womens Research Institute, Pittsburgh, Pennsylvania. 22

The DLK gene is a target for PPARγ-mediated transcriptional activation during both adipogenesis and neurogenesis.
Jean-Philippe Couture, Richard Blouin.
Presenter affiliation: Université de Sherbrooke, Sherbrooke, Canada. 23

Selective modulation of GR can distinguish between transrepression of NF-κB and AP-1
Karolien De Bosscher, Ilse M. Beck, Nadia Bougarne, Debby Bracke, Ine Vanherpe, Wim Vanden Berghe, Guy Haegeman.
Presenter affiliation: University of Gent, Gent, Belgium. 24

Increased glucocorticoid receptor expression and activity mediate the LPS resistance of SPRET/Ei mice
Lien Dejager, Iris Pinheiro, Leen Puimege, Ye-Dong Fan, Claude Libert.
Presenter affiliation: VIB, Zwijnaarde (Ghent), Belgium; UGent, Zwijnaarde (Ghent), Belgium. 25

GRIP1 as a target for glucocorticoid-induced phosphorylation
Jana Dobrovolna, Yurii Chinenov, Inez Rogatsky.
Presenter affiliation: Hospital for Special Surgery, New York, New York. 26

Nur77-dependent deregulation of PPARγ2 in fasted white adipose tissue
Kalina Duszka, Anne M. Krogsdam, Juliane Bogner-Strauss, Andreas Prokesch, Zlatko Trajanoski.
Presenter affiliation: Innsbruck Medical University, Innsbruck, Austria. 27

The role ERK1/2 and androgen receptor phosphorylation at serine 81 in the progression to castrate resistant prostate cancer.
Pamela McCall, Claire Orange, Morag Seywright, Mark Underwood, Joanne Edwards.
Presenter affiliation: University of Glasgow, Glasgow, United Kingdom. 28

Genome-wide effects of RNAi-mediated knockdown of steroidogenic factor-1 in adrenocortical cells
Anna Ehrlund, Cecilia Williams, Jan-Åke Gustafsson, Eckardt Treuter.
Presenter affiliation: Karolinska Institutet, Stockholm, Sweden. 29

PGC1α-independent regulation of VEGF and vascularization by ERRγ
Vihang A. Narkar, Weiwei Fan, Michael Downes, Ruth T. Yu, Johan W. Jonker, Ronald M. Evans.
Presenter affiliation: Howard Hughes Medical Institute, The Salk Institute, La Jolla, California. 30

FoxO1 regulates Tlr4 inflammatory pathway signaling in macrophages
WuQiang Fan, Jerrold M. Olefsky.
Presenter affiliation: University of California, San Diego, La Jolla, California. 31

USP7/HAUSP stabilizes Tip60 in adipogenesis
Yuan Gao, Olivier van Beekum, Arjen Koppen, Nicole Hamers, Aukje Veenstra, Ruud Berger, Aart G. Jochemsen, Arjan B. Brenkman, Huib Ovaa, Eric Kalkhoven.
Presenter affiliation: UMC Utrecht, Utrecht, Netherlands; Netherlands Metabolomics Centre, Utrecht, Netherlands. 32

Cactin, a novel regulator of TLR signalling pathways
Siobhan Gargan, Paola Atzei, Paul Moynagh.
Presenter affiliation: Institute of Immunology, Maynooth, Republic of Ireland. 33

Expression profiling defines a universal glucocorticoid response program in airway smooth muscle, including non-inflammatory targets that alter airway function
Kiriko Masuno, Sarah K. Sasse, Mukesh Jain, Anthony N. Gerber.
Presenter affiliation: National Jewish Health, Denver, Colorado. 34

Genome-wide binding of estrogen receptor α in response to bisphenol A, genistein or estradiol exposure is cell-type specific and associates with FOXA1 binding
Jason Gertz, Timothy E. Reddy, Katherine E. Varley, Rebekka O. Sprouse, Kimberly M. Newberry, Stephanie L. Parker, Richard M. Myers.
Presenter affiliation: HudsonAlpha Institute, Huntsville, Alabama. 35

JMJD3 regulates *Bcl-2* transcription in ERα-dependent breast cancer cells
Amy Svotelis, Gabrielle Huppé, Stéphanie Bianco, Nicolas Gévry.
Presenter affiliation: Université de Sherbrooke, Sherbrooke, Canada. 36

RBCK1 regulates proliferation in breast cancer cells potentially through control of ERα gene expression
Nina Gustafsson, Chunyan Zhao, Jan-Åke Gustafsson, Karin Dahlman-Wright.
Presenter affiliation: Karolinska Institute, Huddinge, Sweden. 37

Plasma cholesterol lowering by FXR agonist Px20350 is likely due to increased export as well as decreased uptake of cholesterol
Eva Hambruch, Thomas Schlueter, Ulrich Abel, Michael Burnet, Ulrich Deuschle, Claus Kremoser.
Presenter affiliation: Phenex Pharmaceuticals AG, Heidelberg, Germany. 38

Potentiation of glucocorticoid signaling by chloroquine via inhibition of lysosomes
Yuanzheng He, Yong Xu, Chenghai Zhang, Xiang Gao, H. Eric Xu.
Presenter affiliation: Van Andel Institute, Grand Rapids, Michigan. 39

Orphan receptor NR2F6 represses autoimmunity
Natascha Hermann-Kleiter, Thomas Gruber, Gottfried Baier.
Presenter affiliation: Medical University, Innsbruck, Austria. 40

Her2-mediated down-regulation of SMRT in tamoxifen-resistant breast cancer
Kuo-Sheng Hsu, Hung-Ying Kao.
Presenter affiliation: Case Western Reserve University, Cleveland, Ohio. 41

Genome wide analysis of factors affecting androgen receptor function reveals new therapeutic targets in prostate cancer
Keren Imberg, Susan Ha, Susan K. Logan, Michael J. Garabedian.
Presenter affiliation: NYU School of Medicine, New York, New York. 42

Transcriptional Mediator subunit MED1 in stromal cells is involved in hematopoietic stem/progenitor cell support through VDR- and Runx2-mediated osteopontin expression
Ruri Ishino, Akiko Sumitomo, Norinaga Urahama, Robert G. Roeder, Mitsuhiro Ito.
Presenter affiliation: Kobe University Graduate School of Health Sciences, Kobe, Japan; The Rockefeller University, New York, New York. 43

Development of a novel pharmacokinetic approach for the use of glucocorticoids in the treatment of inflammatory diseases
Maryam Jangani, David W. Ray, Rachelle P. Donn.
Presenter affiliation: University of Manchester, United Kingdom. 44

Williams-Beuren syndrome chromosome region 22 (WBSCR22) is a novel modulator of glucocorticoid sensitivity.
Maryam Jangani, Laura M. Green, Laura Elsby, David W. Ray, Rachelle P. Donn.
Presenter affiliation: University of Manchester, United Kingdom. 45

BDNF and glucocorticoid receptor signaling and crosstalk
Freddy D. Jeanneteau, Marcus Lambert, Thomas A. Neubert, Francis S. Lee, Michael J. Garabedian, Moses V. Chao.
Presenter affiliation: NYU-Skirball Institute of Biomolecular Medicine, New York, New York. 46

The actin-binding protein, α actinin alpha 4 (ACTN4), is a nuclear receptor coactivator that is required for estrogen receptor-mediated transcriptional activation in MCF-7 breast cancer cells
Simran Khurana, Sharmistha Chakraborty, Xiwen Cheng, Yu-Ting Su, Hung-Ying Kao.
Presenter affiliation: Case Western Reserve University, Cleveland, Ohio. 47

Targeting EGFR-associated signaling pathways in non-small cell lung cancer cells—Implication in radiation response
In Ah Kim.
Presenter affiliation: Seoul National University, Seoul, South Korea. 48

Modeling of the aryl hydrocarbon receptor ligand binding pocket and identification of new ligands by virtual ligand screening
Daniel C. Koch, William H. Bisson, Edmond F. O'Donnell, Prasad R. Kopparapu, Nancy I. Kerkvliet, Ruben Abagyan, Robert L. Tanguay, Siva K. Kolluri.
Presenter affiliation: Oregon State University, Corvallis, Oregon. 49

In silico analysis in combination with high-throughput interactomics identifies putative novel brown/white specific PPARγ coregulators
Arjen Koppen, Rene Houtman, Eric Kalkhoven.
Presenter affiliation: University Medical Centre Utrecht, Utrecht, Netherlands. 50

Hairless—A novel transcriptional regulator of adipocyte differentiation
Susann Kumpf, Arne Ittner, Romeo Ricci.
Presenter affiliation: ETH Zurich, Zurich, Switzerland. 51

Mechanisms of glucocorticoid-induced muscle atrophy and insulin resistance
Taiyi Kuo, Michelle J. Lew, Oleg Mayba, Terry Speed, Jen Chywan Wang.
Presenter affiliation: University of California, Berkeley, Berkeley, California. 52

Modulation of glucocorticoid receptor transcriptional activity via BDNF-dependent phosphorylation
W. Marcus Lambert, Freddy Jeanneteau, Thomas Neubert, Moses V. Chao, Michael J. Garabedian.
Presenter affiliation: NYU School of Medicine, New York, New York. 53

Liver X receptor (LXR) regulates human adipocyte lipolysis
Britta M. Stenson, Mikael Rydén, Nicolas Venteclef, Dominique Langin, Peter Arner, Jurga Laurencikiene.
Presenter affiliation: Karolinska Institute, Stockholm, Sweden. 54

High throughput phage display identified novel ERβ interactors
Thien Le, Matthew Walker, Benjamin Casterline, Geoffrey Greene.
Presenter affiliation: University of Chicago, Illinois. 55

The role of the retinoid x receptors in stem cell differentiation
Melanie C. Le May, Chenchen Hou, Natascha Lacroix, Hymn Mach, Qiao Li.
Presenter affiliation: University of Ottawa, Ottawa, Canada. 56

PPARγ2 controls bone mass, marrow mesenchymal stem cell differentiation and energy metabolism in bone
Beata Lecka-Czernik, Clifford Rosen, Masanobu Kawai, Piotr Czernik, Amrei Krings.
Presenter affiliation: University of Toledo Medical Center, Toledo, Ohio. 57

Antiinflammatory effects of a novel agonist ligand for the nuclear receptor LRH-1 in inflammatory bowel disease
Jae M. Lee, Jennifer Mamrosh, Charity M. Mills, David D. Moore.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 58

The coactivator SRC-1 is an essential coordinator of hepatic glucose production
Jean-Francois Louet, Atul Chopra, Jorn V. Sagen, Brian York, Chris B. Newgard, Bert W. O'Malley.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 59

WEDNESDAY, September 1—4:30 PM

Wine and Cheese Party

SESSION 4 INFLAMMATION AND METABOLIC DISEASE

Chairperson: **B. Desvergne,** University of Lausanne, Switzerland

Non-invasive imaging of pancreatic inflammation in models and patients
Diane J. Mathis.
Presenter affiliation: Harvard Medical School, Boston, Massachusetts. 60

Cryo-electron microscopy structure of the 100kDa full nuclear receptor RXR/VDR heterodimer complex with its target DNA
Igor Orlov, Natacha Rochel, Dino Morals, Bruno Klaholz.
Presenter affiliation: IGBMC, Illkirch, France. 61

Inflammatory links between obesity, diabetes and energy expenditure
Alan Saltiel
Presenter affiliation: University of Michigan, Ann Arbor. 62

Identification of novel targets for PPARγ using transcriptional promoter ontology
Johan W. Jonker, Michael Downes, Ronald M. Evans.
Presenter affiliation: Salk Institute, La Jolla, California; University Medical Center Groningen, Groningen, Netherlands. 63

GPR120 is an Omega-3 fatty acid receptor mediating potent anti-inflammatory and insulin sensitizing effects
Da Young Oh, Saswata Talukdar, Jerrold M. Olefsky.
Presenter affiliation: University of California, San Diego, La Jolla, California. 64

Structural mechanisms of signal integration in RXR heterodimers
John Bruning, Andrew I. Shulman, German Gil, Nowak Jason, David J. Mangelsdorf, Michael Chalmers, Patrick R. Grififn, Kendall Nettles.
Presenter affiliation: Scripps Research Institute, Jupiter, Florida. 65

Metabolic flexibility, stress and inflammation
Philipp E. Scherer.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 66

THURSDAY, September 2—9:00 AM

SESSION 5 LIGANDS AND COFACTORS

Chairperson: **R.M. Evans,** The Salk Institute, La Jolla, California

Mechanistic and physiological roles of coregulators in transcriptional regulation by steroid receptors
Michael R. Stallcup.
Presenter affiliation: University of Southern California, Los Angeles, California. 67

In vitro identification of selective androgen receptor modulators using an AR conformation change assay
Jeremy O. Jones.
Presenter affiliation: City of Hope Beckman Research Institute, Duarte, California. 68

Genome-wide chromatin remodeling during adipogenesis
Susanne Mandrup.
Presenter affiliation: University of Southern Denmark, Odense M, Denmark. 69

A distinct class of nuclear receptor alternate site modulators (NRAMs) that target a novel androgen receptor regulatory mechanism involving FKBP52 and β-Catenin
Johanny Meneses De Leon, Aki Iwai, Jane Trepel, Clementine Feau, R. Kip Guy, Robert Fletterick, Leaonard M. Neckers, Marc B. Cox.
Presenter affiliation: University of Texas at El Paso, El Paso, Texas. 70

Variation in glucocorticoid receptor binding sequence and its effect on structure
Miles A. Pufall, Samantha Cooper, Lisa Watson, Keith R. Yamamoto.
Presenter affiliation: University of California-San Francisco, San Francisco, California; University of Iowa-Carver College of Medicine, Iowa City, Iowa. 71

NR coactivators—Physiology and disease
Bert O'Malley.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 72

SESSION 6 POSTER SESSION II

Identification and mechanism of decanoic acid as a natural modulating ligand of PPARγ
Raghu R. V. Malapaka, Sok Kean Khoo, Yong Xu, James H. Resau, Eugene Chen, Eric Xu.
Presenter affiliation: Van Andel Institute, Grand Rapids, Michigan. 73

Multiple nuclear export signals in the thyroid hormone receptor—Implications for resistance to thyroid hormone syndrome
Manohara S. Mavinakere, Jeremy Powers, Vincent R. Roggero, Lizabeth A. Allison.
Presenter affiliation: College of William & Mary, Williamsburg, Virginia. 74

The role of androgen receptor phosphorylation in castrate resistant prostate cancer
Pamela McCall, Claire Orange, Morag Seywright, Joanne Edwards.
Presenter affiliation: University of Glasgow, Glagow, United Kingdom. 75

Regulation of androgen receptor mediated transcription by RPB5 binding protein (RMP/URI)
Paolo Mita, Susan Ha, Susan K. Logan.
Presenter affiliation: New York University School of Medicine, New York, New York. 76

Screening patients with severe insulin resistance identifies a novel FPLD-associated PPARγ mutation (Y151C)
Maartje E. Visser, Elise Kropman, Mariëtte E. Kranendonk, Arjan Koppen, Erik S. Stroes, Eric Kalkhoven, Houshang Monajemi.
Presenter affiliation: UMC Utrecht, Utrecht, Netherlands. 77

Ligand-independent transcriptional actions of human ERβ splice variants on ERE, AP-1 and human arginine vasopressin (hAVP) promoters in neuronal cells
Natasha N. Mott, Toni R. Pak.
Presenter affiliation: Loyola University Medical Center, Maywood, Illinois. 78

Estrogen: estrogen receptor alpha activated negative regulators of growth factor signaling in breast cancer.
Harikrishna Nakshatri, Hitesh Appaiah, AJ Sood, Jennifer Sondhi, Sunil Badve, Poornima Bhat-Nakshatri.
Presenter affiliation: Indiana University School of Medicine, Indianapolis, Indiana. 79

Dexamethasone (DEX), cortisol (COR), and aldosterone (ALD) generate common and unique patterns of gene expression in human trabecular meshwork cells
Alissar Nehme, Stefanie J. Kirwin, W D. Stamer, Jeffrey L. Edelman.
Presenter affiliation: Allergan Inc, Irvine, California. 80

AHR-mediated immunosuppression by alternative AHR ligands—A novel strategy for treatment of autoimmune disease
Ed F. O'Donnell, Prasad Kopurapu, Diana Rohlman, Dan Koch, Siva K. Kolluri, Nancy I. Kerkvliet.
Presenter affiliation: Oregon State University, Corvallis, Oregon. 81

Estrogen receptor β is a potential therapeutic target to suppress lymphoid tumor growth
Konstantin Yakimchuk, Maryam Iravani, Stefan Nilsson, Mikael Jondal, Sam Okret.
Presenter affiliation: Karolinska Institutet, Huddinge, Sweden. 82

Androgen- and glucocorticoid-induced activation of FKBP51 locus through long-range interactions
Ville Paakinaho, Harri Makkonen, Tiina Jääskeläinen, Jorma Palvimo.
Presenter affiliation: University of Eastern Finland, Kuopio, Finland. 83

Identification of a novel estrogen responsive estrogen receptor α (ESR1) variants in the pregnant uterus.
Clifton R. Moore, Steve N. Caritis, Christina M. Kachulis, Jennifer C. Condon, Pancharatnam Jeyasuria.
Presenter affiliation: University of Pittsburgh, School of Medicine, Pittsburgh, Pennsylvania. 84

Opposite roles of additional sex comb-like (ASXL) 1 and 2 in adipogenesis via differential regulation of PPARγ
Ui-Hyun Park, Na-ra Shin, Eun-Joo Kim, Soo-Jong Um.
Presenter affiliation: Sejong University, Seoul, South Korea. 85

NHR-49 influences mitochondrial physiology by regulating distinct aspects of lipid metabolism
Pranali P. Pathare, Marc Van Gilst.
Presenter affiliation: Fred Hutchinson Cancer Research Center, Seattle, Washington. 86

Warfarin enantiomers interactions with Pregnane X nuclear (PXR) receptor in gene regulation of cytochrome P450 CYP3A4 enzyme
Alice Rulcova, Lucie Svecova, Radim Vzal, Michal Bitman, Zdenek Dvorak, Petr Pavek.
Presenter affiliation: Charles University in Prague, Hradec Kralove, Czech Republic. 87

Glucocorticoid receptor transcriptional regulation of the circadian clock gene *PER1*
Marlisa L. Pillsbury, Keith R. Yamamoto.
Presenter affiliation: University of California, San Francisco, San Francisco, California. 88

LXR activation induces Arginase 1 expression in macrophages
Benoit Pourcet, Ines Pineda-Torra.
Presenter affiliation: University College London, London, United Kingdom. 89

Effects of free fatty acids on the expression of PGC1-α and insulin signaling in skeletal muscle cells
Larysa Yuzefovych, Lyudmila Rachek.
Presenter affiliation: University of South Alabama, Mobile, Alabama. 90

A network of regulatory elements directs the glucocorticoid receptor to express Period 1 at low doses of corticosteroid
Timothy E. Reddy, Jason Gertz, Katherine E. Varley, Michael J. Garabedian, Richard M. Myers.
Presenter affiliation: HudsonAlpha Institute for Biotechnology, Huntsville, Alabama. 91

Aging the SMRT way—Regulation of mitochondrial oxidative metabolism and aging related metabolic deterioration by nuclear receptor co-repressor SMRT
Shannon M. Reilly, Prerna Bhargava, Sihao Liu, Matthew R. Gangl, Russell Nofsinger, Cem Gorgun, Lu Qi, Frank Hu, Chih-Hao Lee.
Presenter affiliation: Harvard School of Public Health, Boston, Massachusetts. 92

Interplay of tumour-derived IL-6 with nuclear receptors in the activation of BAT in cancer cachexia/anorexia syndrome
Maria Tsoli, Melissa Moore, Ryland Taylor, Arran Painter, Stephen Clarke, Graham Robertson.
Presenter affiliation: ANZAC Research Institute, Concord RG Hospital, Australia. 93

Synergistic growth inhibition by retinoic acid and Herceptin™ in HER2/RARA co-amplified breast cancer cells
Marieke Rozendaal, Slim Fourati, Sylvie Mader.
Presenter affiliation: University of Montreal, Montréal, Canada. 94

Thyroid hormone induces the expression of Serum and Glucocorticoid regulated kinase 1(SGK1) in HepG2 hepatoma cell line
Prabodh Sadana.
Presenter affiliation: Northeastern Ohio Universities Colleges of Medicine and Pharmacy, Rootstown, Ohio. 95

Dual function of FoxA1 in regulation of androgen receptor binding to chromatin
Biswajyoti Sahu, Marko Laakso, Sampsa Hautaniemi, Olli A. Jänne.
Presenter affiliation: University of Helsinki, Helsinki, Finland. 96

Transient, non-genomic action of the glucocorticoid receptor limits gap junction intercellular communication and neural progenitor cell proliferation
Ranmal A. Samarasinghe, Marcia Lewis, Donald B. DeFranco.
Presenter affiliation: University of Pittsburgh, Pittsburgh, Pennsylvania. 97

The gene regulatory response of mouse aortic cells to estrogen is linked to a distinct distribution of ERα on chromatin
Francesca K. Gordon, Michelle Jamongjit, Richard Karas, Michael Mendelsohn, Gavin R. Schnitzler.
Presenter affiliation: Tufts Medical Center, Boston, Massachusetts. 98

The testosterone metabolite 5α-androstane-3β, 17β-diol (3β-diol) leads to differential ERα and β occupancy of the oxytocin (OT) promoter, a stress-related gene
Dharmendra Sharma, Robert J. Handa, Rosalie M. Uht.
Presenter affiliation: Health Institutes of Texas, Fort Worth, Texas. 99

Integration of IL-1β and androgen receptor signaling—Characterizing two distinct intersections
Julia A. Staverosky, Susan Ha, Rachel Ruoff, Susan K. Logan.
Presenter affiliation: New York University School of Medicine, New York, New York. 100

Enteric bacterial regulation of Vitamin D receptor signaling in intestinal inflammation
Shaoping Wu, Jun Sun.
Presenter affiliation: University of Rochester, Rochester, New York. 101

Purification and identification of RORγt co-regulators in T helper cells
Ichiro Takada, Yogiashi Yoshiko, Akihiko Yoshimura.
Presenter affiliation: Keio University, School of Medicine, Tokyo, Japan. 102

Identification and functional analysis of AP2γ as a novel transcriptional cofactor of estrogen receptor in breast cancer
Si Kee Tan, Zhen Huan Lin, Cheng Wei Chang, Kern Rei Chng, You Fu Pan, Wing Kin Sung, Edwin Cheung.
Presenter affiliation: Genome Institute of Singapore, A*STAR (Agency for Science, Technology and Research), Singapore. 103

Cancer Cachexia syndrome—Impact of tumour-derived IL-6 on nuclear receptors and circadian regulation of metabolic pathways in livers of cachectic mice
Ryland Taylor, Arran Painter, Maria Tsoli, Stephen Clarke, Graham Robertson.
Presenter affiliation: ANZAC Research Institute, Concord RG Hospital, Australia. 104

PPARgamma in regulation of aortic stiffness and pulse pressure
Hao-Chih Tai, Ju-Yi Chen, Pei-Jane Tsai, Yau-Sheng Tsai.
Presenter affiliation: National Cheng Kung University, Tainan, Taiwan. 105

Foxl2 interacts with ER in female sex maintenance
Henriette N. Uhlenhaut, Robin Lovell-Badge, Ronald M. Evans, Mathias Treier.
Presenter affiliation: Max Delbrück Center for Molecular Medicine, Berlin, Germany. 106

Genome wide analysis of vdr binding sites in human monocytes
Sami Vaisanen, Sami Heikkinen, Petri Pehkonen, Sabine Seuter, Merja Matilainen, Carsten Carlberg.
Presenter affiliation: University of Eastern Finland, Kuopio, Finland. 107

PPARγ inhibits allogenic CTL cytotoxicity
Andreas von Knethen, Martina V. Schmidt, Bernhard Bruene.
Presenter affiliation: Goethe-University Frankfurt, Frankfurt, Germany. 108

Dose-dependent effects of progestins on progesterone receptor activity and target gene expression—Less is more?
Hilary E. Wade, Donald P. McDonnell.
Presenter affiliation: Duke University Medical Center, Durham, North Carolina. 109

RAC3 is a novel pro-migratory coactivator of ERα with links to breast cancer progression
Matthew P. Walker, Maomao Zhang, Thien P. Le, Muriel Laine, Geoffrey L. Greene.
Presenter affiliation: University of Chicago, Chicago, Illinois. 110

Specific phosphorylation of coactivator Mediator 1 coding for UBE2C locus looping leads to androgen receptor negative prostate cancer growth
Zhong Chen, Chunpeng Zhang, Dayong Wu, Anna Rorick, Qianben Wang.
Presenter affiliation: Ohio State University, Columbus, Ohio. 111

The reduction of LXR activity by ezetimibe improves the fatty liver and diabetes
Mitsuhiro Watanabe, Taichi Sugizaki, Yasushi Horai, Kokichi Morimoto, Eri Arita, Hiroshi Itoh.
Presenter affiliation: Keio University, Tokyo, Japan. 112

Tissue-specific in vivo roles of nuclear receptor cofactor MED1
Xiaoting Zhang.
Presenter affiliation: University of Cincinnati, Cincinnati, Ohio. 113

SESSION 7 CANCER

Chairperson: **C. Glass,** University of California, San Diego

Identification and exploitation of targetable proteins/processes in the AR signaling pathway involved in prostate cancer pathogenesis
Donald McDonnell.
Presenter affiliation: Duke University Medical Center, Durham, North Carolina. 114

Constitutively active androgen receptor splice variants expressed in castration-resistant prostate cancer require full-length androgen receptor
Philip A. Watson, Yinan F. Chen, Minna D. Balbas, John Wongvipat, Nicholas D. Socci, Agnes Viale, Kwanghee Kim, Charles L. Sawyers.
Presenter affiliation: Memorial Sloan-Kettering Cancer Center, New York, New York. 115

Growth regulation by androgen receptor in estrogen receptor-negative breast cancer
Myles Brown.
Presenter affiliation: Dana-Farber Cancer Institute, Boston, Massachusetts. 116

An epigenetic cell-based screen identifies a novel small molecule with specific anti-cancer activity which modulates transcription
Jianjun Chang, Anne Best, Diana Varghese, Elisabeth D. Martinez.
Presenter affiliation: UT Southwestern Medical Center, Dallas, Texas. 117

Androgen receptor function is regulated by histone demethylases: Implication for prostate cancer
Eric Metzger, Axel Imhof, Dharmeshkumar Patel, Philip Kahl, Nicolaus Friedrichs, Judith M. Müller, Holger Greschik, Jutta Kirfel, Thomas Günther, Reinhard Buettner, Roland Schüle.
Presenter affiliation: University of Freiburg, Germany. 118

ChIP-Seq based characterization of genes differentially responding to AR ligands in advanced prostate cancer cells
Ling Cai, Azra Krek, Nick Socci, Charles Sawyers.
Presenter affiliation: Memorial Sloan-Kettering Cancer Center, New York, New York. 119

Novel roles for nuclear receptors in breast cancer epithelium and stroma
Vincent Giguère, Geneviève Deblois, Lillian J. Eichner, Xingxing Liu.
Presenter affiliation: McGill University, Montreal, Canada. 120

FRIDAY, September 3—9:00 AM

SESSION 8 MECHANISMS OF INSULIN RESISTANCE AND ADIPOGENESIS

Chairperson: **J.M. Olefsky,** University of California, San Diego

Skeletal muscle metabolism—AMPK and beyond
Laurie Goodyear.
Presenter affiliation: Joslin Diabetes Center, Boston, Massachusetts. 121

Glucocorticoid receptor and triglyceride homeostasis
Nora E. Gray, Chi-Yi Yu, Oleg Mayba, Joyce V. Lee, Terry Speed, Jen-Chywan Wang.
Presenter affiliation: University of California Berkeley, Berkeley, California. 122

A human PPARγ mutation causes inherited diabetes through a unique mechanism of action
Olga I. Astapova, Philippe M. Campeau, Claude Gagne, Robert A. Hegele, Todd A. Leff.
Presenter affiliation: Wayne State University School of Medicine, Detroit, Michigan. 123

Coactivators and disease—Ablation of SRC-3 phenocopies a myopathy resembling human CACT deficiency
Brian York, Erin L. Reineke, Jorn V. Sagen, Jean-Francois Louet, Atul Chopra, Bert W. O'Malley.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 124

Epigenomic regulation of circadian rhythm and metabolism by nuclear receptors
Mitchell A. Lazar.
Presenter affiliation: University of Pennsylvania School of Medicine, Philadelphia, Pennsylvania. 125

Homeostatic levels of SRC-2 and SRC-3 promote early human adipogenesis
Sean M. Hartig, Bin He, Benjamin M. Buehrer, Michael A. Mancini.
Presenter affiliation: Baylor College of Medicine, Houston, Texas. 126

Metabolomics applied to understanding of nuclear receptor biology and metabolic disease
Christopher Newgard.
Presenter affiliation: Duke University Medical Center, Durham, North Carolina. 127

FRIDAY, September 3—2:00 PM

SESSION 9 INFLAMMATION II

Chairperson: **S. Mandrup,** University of Southern Denmark, Odense

Nuclear receptor repression pathways that regulate microglia and astrocyte activation
Christopher K. Glass.
Presenter affiliation: University of California, San Diego. 128

Protective role of endothelial PPARγ in atherosclerosis
Ii-Jung Tai, Erin Dunn, Grant Barish, Rachel Richards, Audrey Black, Linda Curtiss, Ron Evans.
Presenter affiliation: Salk Institute, La Jolla, California. 129

Inflammation, endoplasmic reticulum stress, and metabolic control
Gokhan Hotamisligil.
Presenter affiliation: Harvard School of Public Health, Boston, Massachusetts. 130

A dual action of glucocorticoids on the type I interferon network
Jamie R. Flammer, Megan A. Kennedy, Yurii Chinenov, Lionel B. Ivashkiv, Inez Rogatsky.
Presenter affiliation: Hospital for Special Surgery, New York, New York; Weill Medical College of Cornell University, New York, New York. 131

Deletion of nuclear receptor corepressor protein (NCOR) in adipose tissue improves systemic insulin resistance in diet-induced obese mice
Pingping Li, Jianfeng Xu, Min Lu, Hiroyasu Yamamoto, Johan Auwerx, Dorothy D. Sears, Saswata Talukdar, David Patsouris, Miriam Scadeng, Jachelle M. Ofrecio, Sarah Nalbandian, Jerrold M. Olefsky.
Presenter affiliation: University of California San Diego, San Diego, California. 132

Identification of STAT6 as a required licensing factor for PPARγ-regulated gene expression in macrophages and dendritic cells
Laszlo Nagy.
Presenter affiliation: University of Debrecen, Hungary. 133

Dynamic exchange prevents receptor competition on regulatory elements
Ty C. Voss, R Louis Schiltz, Myong-Hee Sung, Thomas A. Johnson, Sam John, Gordon L. Hager.
Presenter affiliation: NCI, National Institutes of Health, Bethesda, Maryland. 134

Lessons from PPARγ null mice, from development to metabolism
Béatrice Desvergne, Laure Quignodon, Karim Nadra, Jean-Gaël Diserens, Chiara Sardella.
Presenter affiliation: University of Lausanne, Lausanne, Switzerland. 135

FRIDAY, September 3—6:00 PM

CONCERT
Grace Auditorium

Di Wu, piano

Praised in The Wall Street Journal as “a most mature and sensitive pianist,” Chinese-born Di Wu’s reputation as an elegant yet exciting musician continues to grow, and 2009 has been a banner year: During the spring and summer she made her New York Alice Tully Hall recital debut as winner of Juilliard’s William Petschek Piano Debut Recital Award, was named Artist of the Month by MusicalAmerica.com, and was awarded a Vendome Virtuosi prize at Lisbon’s prestigious Vendome Competition as well as one of the coveted prizes at the Thirteenth Van Cliburn International Piano Competition. In September she made her Philadelphia Orchestra debut at a gala benefit concert under Charles Dutoit.
Ms. Wu made her professional debut at 14 with the Beijing Philharmonic, since then she has toured widely in Asia, Europe and the United States, where her recent orchestral engagements have included appearances with Washington’s National Symphony, the Pittsburgh Symphony, the New Jersey Symphony Orchestra, and twice in Carnegie Hall with the New York Pops. During the 2009-10 season, Ms. Wu is scheduled for a 40-concert tour which will take her from California to Germany.

Di Wu came to the United States in 1999 to study at the Manhattan School of Music with Zenon Fishbein. From 2000 to 2005 she studied at the Curtis Institute with Gary Graffman, subsequently earning a Master of Music degree at The Juilliard School under Yoheved Kaplinsky and, in 2009, an Artist Diploma under the guidance of Joseph Kalichstein and Robert McDonald. Ms. Wu is currently represented by Astral Artists.

FRIDAY, September 3

BANQUET

Cocktails 7:00 PM Dinner 7:45 PM

SATURDAY, September 4—9:00 AM

SESSION 10 CHROMATIN AND TRANSCRIPTION

Chairperson: **M. Stallcup**, University of Southern California, Los Angeles

Genomic analyses of estrogen-regulated transcription reveal new facets of the estrogen signaling pathway
W. Lee Kraus, Nasun Hah, Charles G. Danko, Leighton Core, John T. Lis.
Presenter affiliation: University of Texas Southwestern Medical Center, Dallas, Texas. 136

The p23 molecular chaperone and GCN5 acetyltransferase cooperatively modulate the stability of protein-DNA complexes
Elena Zelin, Frank J. Echtenkamp, Ellinor Oxelmark, Joyce I. Woo, Michael J. Garabedian, Brenda J. Andrews, Brian C. Freeman.
Presenter affiliation: University of Illinois, Urbana-Champaign, Urbana, Illinois. 137

TLE3 is a dual function transcriptional coregulator of adipogenesis
Peter Tontonoz.
Presenter affiliation: University of California, Los Angeles. 138

Sumoylation of *C. elegans* nuclear receptor NHR-25 promotes proper organogenesis
Jordan D. Ward, Nagagireesh Bojanala, Teresita Bernal, Kaveh Ashrafi, Marek Jindra, Masako Asahina, Keith R. Yamamoto.
Presenter affiliation: UCSF, San Francisco, California. 139

Dynamics of nuclear receptor interactions at global regulatory elements
Gordon L. Hager.
Presenter affiliation: NCI, National Institutes of Health, Bethesda, Maryland. 140

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NUCLEAR RECEPTOR MODULATORS (NURMS) IN WORMS

Steven A Kliewer, David J Mangelsdorf

University of Texas Southwestern Medical School, Departments of Pharmacology, Molecular Biology, and Howard Hughes Medical Institute, Dallas, TX, 75390

The ability to regulate nutrient metabolism under conditions of excess (i.e., after a big meal) or deprivation (i.e., starvation) is a physiologic process that coincided with the evolution of nuclear receptors in all multi-cellular organisms. In mammals, nuclear receptor systems have evolved to respond to cholesterol, bile acids, and fatty acids and thereby govern nutrient metabolism in the fed and fasted state. Recently, we have discovered that this process is conserved in nematodes, and this has led to the discovery that nuclear receptor regulated pathways may be exploited therapeutically to target two unexpectedly related human diseases: type 2 diabetes and nematode parasitism.

PPARγ AND THE ANTI-DIABETIC PPARγ LIGANDS: A NEW LOOK AT AN OLD FRIEND

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The nuclear receptor PPARγ is the dominant regulator of fat cell differentiation and development. Expression and agonist activation of PPARγ is sufficient for adipose cell differentiation in most fibroblastic cells, and loss of function blocks fat development. Humans with dominant-negative mutations in PPARγ have a form of lipodystrophy with severe insulin resistance and hypertension. In addition to these roles in adipose formation, PPARγ is the functioning receptor for the TZD anti-diabetic ligand drugs. These drugs such as rosiglitazone are full agonists for PPARγ. In new work, we show that obesity induced in mice by high-fat feeding activates the protein kinase cdk5 in adipose tissues. This results in phosphorylation of PPARγ at serine 273. This modification of PPARγ does not alter its adipogenic capacity, but leads to dysregulation of a large number of genes whose expression is altered in obesity, including a reduction in the expression of the insulin-sensitizing adipokine, adiponectin. The phosphorylation of PPARγ by cdk5 is blocked by anti-diabetic PPARγ ligands, such as rosiglitazone and MRL24. This inhibition works both *in vivo* and *in vitro*, and surprisingly, is completely independent of classical receptor transcriptional agonism. Similarly, inhibition of PPARγ phosphorylation in obese patients by rosiglitazone is very tightly associated with the anti-diabetic effects of this drug. These data strongly suggest that cdk5-mediated phosphorylation of PPARγ may be involved in the pathogenesis of insulin-resistance, and present an opportunity for development of an improved generation of anti-diabetic drugs through PPARγ.

INTEGRATING METABOLIC CONTROL BY NAD+ SENSORS

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A century after the identification of a co-enzymatic activity for NAD+, NAD+ metabolism has come in the spotlight again due to the potential therapeutic relevance of a set of enzymes whose activity is tightly regulated by the balance between the oxidized and reduced forms of this metabolite. In fact, the actions of NAD+ have been extended from being an oxidoreductase cofactor for single enzymatic activities to acting as a substrate for a wide range of proteins. These include NAD+-dependent sirtuin protein deacetylase, poly(ADP-ribose) polymerases, and transcription factors that affect a large array of cellular functions. Through these effects NAD+ provides a direct link between the cellular redox status and the control of signaling and transcriptional events. Of particular interest within the metabolic/endocrine arena are the recent results, which indicate that the regulation of these NAD+-dependent pathways may have a major contribution to oxidative metabolism and lifespan extension. I will provide an integrated view on how the control of NAD+ production and cycling, as well as its cellular compartmentalization, alters transcriptional pathways via NAD+'s commanding role on cofactor networks that involve and SIRT1, GCN5, and PGC-1α. As such the modulation of NAD+-producing and -consuming pathways have a major physiological impact and hold promise for the prevention and treatment of metabolic disease.

THE NUCLEAR BILE ACID RECEPTOR FXR AND SIRT1 DEACETYLASE FORM A DYNAMIC INTERACTIVE REGULATORY NETWORK CONTROLLING HEPATIC LIPID METABOLISM

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Imbalance in metabolic equilibrium and accumulation of lipid metabolites, bile acids, cholesterol, and fat, play causative roles in metabolic disorders. The nuclear bile acid receptor FXR plays an important role in maintaining lipid levels by activating or repressing its numerous target genes. The NAD+-dependent SIRT1 deacetylase also plays a critical role in cellular metabolism by modulating the activity of key metabolic regulators including PGC-1a. Although both FXR and SIRT1 are critical for hepatic metabolism and activation of these proteins lead to beneficial transcriptional profiling and metabolic outcomes in metabolic disease states, it is not clear whether these two proteins are coordinately regulated. Our published and current studies provide evidence that FXR and SIRT1 positively regulate each others' levels and activities to maintain metabolic homeostasis. FXR activation positively regulates hepatic SIRT1 protein levels by inhibiting expression of the non-coding small microRNA34a (miR-34a), which inhibits SIRT1 by binding to its 3'UTR. Levels of miR-34a are elevated and SIRT1 protein levels are decreased in obese mice, and interestingly, FXR activation by daily treatment for 1 week with GW4064, a synthetic FXR agonist, reverses these effects. It has been shown that SIRT1 levels are substantially reduced in diet-induced obese mice but how SIRT1 levels are reduced remains unclear. Our studies explain, at least in part, decreased SIRT1 protein levels are due to elevated miR34a levels as a result of deregulated FXR activity in obese mice. Conversely, SIRT1 also increases transactivation potential of FXR because acetylation dampens FXR activity by inhibiting heterodimerization with RXRa and binding to DNA/chromatin. Remarkably, FXR acetylation is dramatically elevated in obese mice and SIRT1 activation in these mice by treatment of resveratrol, a natural SIRT1 activator, or adenoviral-mediated overexpression of SIRT1 decreases FXR acetylation levels with beneficial lipid metabolic outcomes. To elucidate functional roles of FXR acetylation, we have identified K157 and K217 as FXR acetylation sites and developed antibodies specific for FXR acetylated at these sites. Using these antibodies, we found that acetylation specifically at K217 is dramatically elevated in obese mice and interaction with SIRT1 is substantially decreased in these mice. We are currently investigating transcriptional and metabolic effects of FXR acetylation at K217 in vivo. Our studies suggest that FXR and SIRT1 constitute an interactive regulatory network dynamically controlling hepatic lipid metabolism and that deregulation of these networks is associated with metabolic disease states. Manipulation of this regulatory network may be useful for treating metabolic diseases, such as fatty liver, obesity, and diabetes (Supported by grants from NIH and the American Diabetes Association).

NHR-49 AS CHIEF ECONOMIST

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In nematodes, NHR-49 acts as a functional homolog of the mammalian PPARs, directing energy storage and expenditure in response to food intake and withdrawal. Using the unique methodological strengths of *C. elegans*, we have been able to comprehensively characterize and dissect the NHR-49 regulatory network. Our studies show that, at the global level, NHR-49 partners with other *C. elegans* NRs to coordinate food supply with reproductive demand, while at the local level NHR-49 directs the assembly and modification of cellular infrastructure to accommodate changes in nutrient accessibility or demand. The cellular functions of NHR-49 are elegantly modeled by the basic principles of supply side economics. In such a model, NHR-49 acts as a chief economist charged with stabilizing global supply and demand. At the local level, NHR-49 works with a team of NR "specialists" that allow NHR-49 to independently control multiple links in the supply side chain. Finally, in addition to subtle modifications, the NHR-49 regulatory mechanism can bring the entire economy of reproduction to a halt if even a simple commodity is missing. This state of reproductive arrest can extend adult longevity by nearly 20-fold, suggesting that the challenges of running a highly productive economy may be causative factors in the aging process. Moving forward, stable isotope labeling strategies provide a unique opportunity to measure economic parameters in a live organism, and to relate these parameters to the genetics of aging and disease.

GENE/ENVIRONMENT INFLUENCE ON SKELETAL MUSCLE INSULIN SENSITIVITY IN TYPE 2 DIABETIC PATIENTS

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Skeletal muscle is an important site of insulin-mediated glucose uptake and defects in this insulin target tissue precede the manifestation of Type 2 diabetes. Our central hypothesis is that activation of insulin-independent pathways to glucose transport in skeletal muscle may overcome the profound impairment in whole body glucose homeostasis associated with Type 2 diabetes. Intensive research efforts have been directed towards understanding the regulation of insulin-dependent and insulin-independent pathways governing glucose metabolism and the factors causing insulin resistance in Type 2 diabetes. Physical exercise/muscle contraction elicits an insulin-independent increase in glucose transport and perturbation of this pathway may bypass defective insulin signaling. To date, the exercise-responsive signaling molecules governing glucose metabolism in skeletal muscle are largely unknown. Epigenetic modification through DNA methylation is implicated in metabolic disease and may play a role in the mechanism by which environmental factors influence metabolic responses in diabetes. Using whole genome promoter methylation analysis of skeletal muscle from normal glucose tolerant and Type 2 diabetic subjects, we identified cytosine hypermethylation of Peroxisome Proliferator-Activated Receptor γ Coactivator-1 α (PGC-1α) in diabetic subjects. Methylation levels were negatively correlated with *PGC-1α* mRNA and mitochondrial DNA (mtDNA). Bisulfite sequencing revealed that the highest proportion of cytosine methylation within *PGC-1α* was found within non-CpG nucleotides. Non-CpG methylation was acutely increased in human myotubes by exposure to tumor necrosis factor-α (TNF-α) or free fatty acids, but not insulin or glucose. Selective silencing of the DNA methyltransferase 3B (*DNMT3B*), but not *DNMT1* or *DNMT3A*, prevented palmitate-induced non-CpG methylation of *PGC-1α* and decreased mtDNA and *PGC-1α* mRNA. We provide evidence for *PGC-1α* hypermethylation, concomitant with reduced mitochondrial content in Type 2 diabetic patients, and link DNMT3B to the acute fatty-acid induced non-CpG methylation of *PGC-1α* promoter. By identifying the molecular mechanisms controlling insulin sensitivity, future development of pharmacological and physiological (exercise and diet) intervention strategies aimed to improve glucose homeostasis may be possible.

PPARα ORCHESTRATES SEXUAL DIMORPHISM IN HEPATIC FUNCTIONS

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Peroxisome proliferator-activated receptors form a small family of three nuclear receptors (PPARα, PPARβ/δ and PPARγ), which act as lipid sensors to modulate gene expression. As most metabolic studies are conducted in male animals, understanding the sex specificity of the underlying molecular pathways has been neglected. For instance, PPARα has been considered as having low activity in female liver, because of the lack of peroxisome proliferation when females are exposed to peroxisome proliferators.

This point of view is now challenged by the observation that there is a sexual dimorphism in PPARα post-translational modification, which plays an active role in gene repression. In female mice, PPARα has broad repressive actions on hepatic genes involved in steroid metabolism and immunity. Using the steroid oxysterol 7α-hydroxylase cytochrome P4507b1 (Cyp7b1) gene as a model, we elucidated the molecular mechanism of this sex-specific PPARα-dependent repression. Initial sumoylation of the ligand-binding domain of PPARα triggered the interaction of PPARα with GA-binding protein alpha (GABPα) bound to the target Cyp7b1 promoter. DNA and histone methyltransferases were then recruited, and the adjacent Sp1-binding site and histones were methylated. These events resulted in loss of Sp1-stimulated expression and thus downregulation of Cyp7b1. Physiologically, this repression conferred on female mice protection against experimentally estrogen-induced intrahepatic cholestasis.

Coregulator recruitment participates in tissue-specific actions of nuclear receptors. To explore whether sexual dimorphism involves coregulators, we analyzed hepatic PPARα protein complexes in both sexes. We found three proteins that interact preferentially with PPARα in female liver. We identified the PPAR interaction motif of one of them and explored its coregulator abilities, which required NADP+, suggesting that PPARα trans-repression is redox-sensitive. One of the processes, which are repressed in females, is isoprenoid synthesis. In support of this finding, co-treatment of hepatoma cells with 17β-estradiol and PPARα ligands diminished isoprenoid synthesis, which was associated with reduced cell proliferation.

Collectively, our data identify PPARα as a key actor of hepatic sexual dimorphism. The ongoing characterization of this function aims to unveil why the female liver is more resistant to inflammation and cancer.

OXYSTEROLS ARE LIGANDS FOR THE ORPHAN NUCLEAR RECEPTOR RORγT, A KEY REGULATOR OF TH17 CELL DEVELOPMENT

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Th17 cells are CD4+ cells that express the pro-inflammatory cytokine IL-17 and have been identified as a distinct T cell lineage involved in mediating inflammation and auto-inflammatory disorders. In human disease, IL-17 has been shown to be elevated in the synovial fluid of rheumatoid arthritis patients as well as in the gut mucosa of Crohn's and ulcerative colitis patients.

The RAR-related orphan receptor (RORγt) is a member of the nuclear receptor superfamily and has been identified as a key transcription factor required for the development of Th17 cells. Nuclear receptors require coactivator binding to activate transcription of their target genes. For many nuclear receptors small molecule ligands bind the ligand binding domain of the receptor and regulate association with the coactivators/corepressors. RORγt has been considered an orphan nuclear receptor as regulation by a small molecule has not been demonstrated.

Utilizing a biochemical coactivator peptide binding assay we have identified a class of cholesterol derivatives with activity specific to RORγt versus RORα and PPARγ. In this assay these molecules perform as agonists and enhance RORγt -LBD protein binding to the coactivator peptide GRIP1. Further validation that these cholesterol derivatives are RORγt ligands was provided by mass spectrometry, a scintillation proximity assay and an X-ray crystal structure of the RORγt ligand binding domain.

In contrast to the agonist activity of oxysterols in the RORγt biochemical assay, these molecules act as inhibitors in a RORγt -dependent reporter assay.

CIRCADIAN TRANSCRIPTIONAL REPRESSORS CRY1 AND CRY2 MEDIATE TRANSREPRESSION BY THE GLUCOCORTICOID RECEPTOR

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Nuclear hormone receptors (NRs) regulate physiology by modulating gene expression in response to lipophilic ligands, including hormones, vitamins, and dietary lipids. Ligand stimulation of NRs can influence the transcription of target genes either positively (transactivation) or negatively (transrepression). Transactivation is mediated by ligand-induced recruitment of co-activators or release of co-repressors while transrepression requires the NR C-terminal domain and unknown accessory factors. We demonstrate that the circadian transcriptional repressors cryptochromes 1 (Cry1) and 2 (Cry2) interact with several nuclear hormone receptors. Studying the interaction of Cry1 and Cry2 with the glucocorticoid receptor (GR) in detail, we found that Cry1 and Cry2 interact with the C-terminus of the GR and are required for most transrepression observed in response to the synthetic GR ligand dexamethasone in mouse embryonic fibroblasts. In addition, dexamethasone-induced transactivation of many genes was increased in Cry-deficient fibroblasts suggesting that cryptochromes oppose transactivation in addition to contributing to transrepression. In mice, genetic loss of Cry1 and/or Cry2 resulted in glucose intolerance and constitutively high levels of circulating corticosterone, suggesting reduced glucocorticoid suppression of the hypothalamic-pituitary-adrenal (HPA) axis coupled with increased sensitivity to the hyperglycemic effects of glucocorticoid-mediated transactivation in the liver. Dexamethasone-induced transcription of pepck was strikingly increased in Cry-deficient livers, while recruitment of GR to the pepck promoter was unaffected by the absence of Cry1 and Cry2. Finally, Cry1-/-;Cry2-/- mice subjected to 8 weeks of chronic dexamethasone treatment exhibited incomplete suppression of circulating corticosterone and greater glucose intolerance compared with wildtype littermates subjected to the same chronic treatment, consistent with a positive shift in the transcriptional response to the synthetic glucocorticoid ligand.

PROMOTER ELEMENTS AND PROTEIN FUNCTION OF THE ATYPICAL COREPRESSOR TNIP1 INDICATE AN EXPRESSION FEEDBACK LOOP

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Nuclear receptor (NR) regulation of transcription is dependent on auxiliary proteins known as coactivators and corepressors. We isolated TNFAIP3 interacting protein 1 (TNIP1) from a human keratinocyte cDNA library and identified it as a corepressor of retinoic acid receptors (RARs). RARs control gene expression in both normal skin and non-skin tissues and are important pharmacologic targets in skin and hematopoietic cancers. TNIP1 interacts with target NRs only in the presence of ligand but represses their activity making it unlike NCoR or SMRT and functionally more like RIP140 and LCoR. Prior to our demonstration of it as a corepressor of ligand-bound RAR, TNIP1 protein was reported i)to shuttle HIV nef and matrix proteins into the nuclei of infected cells and ii)to suppress cytoplasmic signaling leading to NF-kappaB activity. Recently, the human TNIP1 gene locus was reported as strongly associated with the inflammatory skin disease psoriasis through a genome-wide scan. TNIP1's expression beyond skin cells and role in diverse pathways prompted us to investigate genomic elements controlling its expression. To this end, we isolated 6kb of the human TNIP1 promoter and subjected it to physical and functional analysis.
Our study demonstrated the human TNIP1 promoter lacks a TATA box and is increasingly GC-rich approaching its variable transcription start sites. We identified Sp1 regulation of the proximal TNIP1 promoter. Intriguingly, we found potential NR binding sites of differing direct repeats within the distal sequence of the 6kb clone. Among these repeats, we demonstrated functional RAR binding sites through receptor up-regulation of the promoter in reporter constructs, receptor binding in mobility shift assays, receptor occupation of these sites in chromatin immuno-precipitation assays, and retinoid up-regulation of the endogenous gene. Through similar approaches, we found two NF-kappaB sites, one each within proximal and distal promoter regions. Positive control of the TNIP1 promoter by RAR and/or NF-kappaB sets the stage for a regulatory circuit by the very proteins whose functions are repressed by TNIP1. These results may place the regulatory elements of the TNIP1 promoter and the functions of its widely-expressed protein in a negative feedback loop. The constitutive (Sp1) and inducible (RAR, NF-kappaB) expression of TNIP1 could contribute control to NR- and NF-kappaB-mediated gene expression networks in normal physiology of multiple cells and be subject to perturbation in psoriasis and other inflammatory diseases.
Supported by grants from NIAMS and AFPE.

IMAGE-BASED ANALYSIS OF LIGAND-SPECIFIC COREGULATOR RECRUITMENT TO AN ESTROGEN RECEPTOR A-OCCUPIED PROMOTER: AN APPLICATION FOR HIGH CONTENT SCREENING.

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Due to its important role in reproductive biology and regulation of diseases including breast cancer and osteoporosis, estrogen receptor α (ER) is the target of many therapeutic compounds including estrogen mimetics and selective ER modulators (SERMs). Considerable evidence also identifies ER as a probable target for some environmentally prevalent endocrine disrupting chemicals (EDCs) that pose serious risks to human health and ecosystems. There is therefore an ongoing need to improve technologies for the study of ligand-dependent modulation of ER, with a precedent to making these technologies accessible for use in both drug development and EDC testing.

Structural and biochemical studies indicate that ligand-specific regulation of ER involves the induction of conformational changes in the receptor that modify its ability to bind to coregulator proteins and thus regulate transcription in a manner that is tissue specific.
We have created and validated a microscopy-based approach to quantify coregulator recruitment to an ER-occupied promoter locus as part of a platform that is amenable to high content screening (HCS). This system exploits an engineered cell line harboring a microscopically-visible multicopy integration of an ER-regulated promoter (termed the PRL array). We have analyzed the dose-dependent recruitment of transcriptional coregulators RNA polymerase II (Pol II) and steroid receptor coactivator-3 (SRC-3) to the PRL-array following treatment with a small panel of SERMs, at multiple timepoints. When compared to a 30 min treatment with 17β-estradiol (E2), 4H-tamoxifen and Raloxifene both repressed the recruitment of Pol II and SRC3 to ER-occupied PRL-arrays. Conversely, treatment with the estrogen mimetic diethylstilbestrol induced similar levels of Pol II and SRC-3 recruitment to E2. Interestingly, the EDCs bisphenol-A and bisphenol-B both induce the recruitment of SRC-3, but not Pol II, to the ER-occupied PRL-array. Studies are ongoing to extend the panel of coregulators tested, with a particular focus on defining ligand-specific differences in coregulator recruitment that may be useful in predicting the tissue-specific physiological effects of SERMs and EDCs.

CONDITIONAL KNOCKDOWN OF P70S6 KINASE IN LIVER PROTECTS AGAINST HEPATIC STEATOSIS AND SYSTEMIC INSULIN RESISTANCE

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Obesity associated hepatic steatosis is manifested by selective insulin resistance where lipogenesis remains sensitive to insulin, whereas, the ability of insulin to suppress glucose production is impaired. P70 S6 kinase (S6K), the downstream substrate of mammalian target of rapamycin, has been highlighted as a molecular link between obesity and insulin resistance. To elucidate the physiological role of S6K in selective insulin resistance, we have created a mice model of liver specific knockdown of S6K by systemic delivery of adeno-associated virus carrying short hairpin small interference RNA for S6K and examined the effects of S6K deficiency on hepatic steatosis and systemic insulin resistance in C57Bl/6 mice. S6K knockdown in liver (L-S6K-KD) resulted in rapid lowering of blood glucose and fasting plasma insulin concentrations, without changes in food intake or body weight. This improvement was accompanied by enhanced glycogen content in liver. L-S6K-KD mice fed high fat diet showed improved glucose tolerance and insulin sensitivity compared to control. This insulin sensitization took place in liver, skeletal muscle, and adipose tissue as assessed by the hyperinsulinemic-euglycemic clamp technique. Additionally, L-S6K-KD mice showed amelioration of steatosis as demonstrated by triglyceride measurements and Oil Red O staining. This was associated with down-regulation of key lipogenic genes upon refeeding after a 15 hr fasting with a robust increase in insulin signal transduction in liver. Importantly, S6K deficiency mediated a significant increase in epididymal fat mass, under refeeding conditions, which is the opposite to the decrease in liver weight. In conclusion, our results demonstrate the importance of S6K as a modulator of the hepatic response to fasting/refeeding and for the development of hepatic steatosis and systemic insulin resistance in obese mice.

LOSS OF ER-REGULATED MICRORNAS IS FUNDAMENTAL TO HORMONE-INDEPENDENT BREAST CANCER CELLS.

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Breast Cancer is a disease that affects thousands of women every year where death generally occurs due to metastasis and development of resistance to chemotherapeutic agents. A large subset of breast cancer is attributed to the dysregulation of estrogen receptor (ER) signaling. Postmenopausal women have decreased systemic estrogen, which presumably leads to a repressed ER signaling program. Treatment of postmenopausal breast cancer patients with aromatase inhibitors, which further decrease the level of estrogen by inhibiting its production from androgens, leads to an improved response when compared to anti-estrogen therapy. Previous studies have examined gene expression profiles in long-term estrogen deprived (LTED) MCF7 cells, providing clues to how these cancers are regulated. We set out to further characterize these cells by examining microRNA (miRNA) expression in LTED cells. As a means to determine miRNAs that may be essential for the regulation of hormone-independent ER-positive breast cancers, we profiled the expression of 738 miRNAs in MCF7 breast cancer cell lines. We compared the miRNA expression levels of common MCF7 cells to a LTED derivative, MCF7:2A. Of the differentially expressed miRNAs, we discovered two different miRNA clusters that are directly regulated by ER in MCF7 cells. We observed induced ER binding near the primary-miRNAs (pri-miRNAs) that contain these clusters and that LTED cells have less overall basal ER interaction at these cis elements. This corresponds to a decreased expression of these pri-miRNAs in the LTED cells versus MCF7. Furthermore, treatment of MCF7s with fulvestrant, an anti-estrogen, reduces pri-miRNA expression. Predicted targets of these miRNAs include IGF1R and HER2, proteins known to be upregulated in LTED cells. We demonstrate modulation of the expression level of these proteins with the addition of miRNAs to LTED cells. Additionally, we show endogenous association of the mRNAs encoding HER2 and IGF1R with the Ago1 complex in MCF7 cells and reduced association in the LTED cells. These data provide a mechanism by which growth receptors can be induced to express upon estrogen loss in breast cancer. Preliminary analysis of published datasets show a correlation between pri-miRNA expression and metastatic breast cancer where there is decreased miRNA expression in metastatic tumors. This study identifies both ER-regulated miRNAs and a potential postmenopausal breast cancer miRNA signature, which may lead to novel therapy of ER-positive breast cancers in postmenopausal women.

EFFECTS OF THE SELECTIVE GLUCOCORTICOID RECEPTOR MODULATOR COMPOUND A ON HSP70'S EXPRESSION AND FUNCTIONALITY IN THE COMBAT OF INFLAMMATION

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Compound A (CpdA) possesses glucocorticoid receptor (GR)-dependent anti-inflammatory properties. Just like classic GR ligands, CpdA can repress NF-κB-mediated gene expression. However, the CpdA-activated GR is driven to a solely monomeric conformation incapable of mediating conventional glucocorticoid response element-regulated gene expression. As dimeric GR-driven transactivation is linked to most of the detrimental side effects of long-term glucocorticoid therapy, e.g. diabetes, osteoporosis, acquired glucocorticoid insensitivity, the selective GR modulator CpdA may thus exhibit a clear advantage over classic glucocorticoids.
Here, we show that this selective GR modulator, similar to heat shock, can potently upregulate the transcription of heat shock genes Hsp70-1A and Hsp70-1B, the proteins of which are described to play a role in the heat shock-induced anti-inflammatory mechanism. These mRNA-based data were further confirmed via reporter gene analyses. Nevertheless, stimulation of Hsp70 by CpdA or heat shock most likely occurs via alternate mechanisms, as CpdA-instigated Hsp70 promoter activation is GR-dependent and HSF1-independent, whereas heat shock-induced Hsp70 expression occurs in a GR-independent and HSF1-dependent manner. Although CpdA can potently stimulate Hsp70 gene expression, Hsp70 protein levels remain unaffected. Moreover, a pharmacological block of translation points out that CpdA does not need any de novo protein synthesis to exert its anti-inflammatory effects. Strikingly, the anti-inflammatory mechanism of both heat shock and CpdA features reduced IκBα degradation and NF-κB p65 translocation. Finally, the presence of the Hsp70 as a chaperone appears to be pivotal for the CpdA-mediated inflammatory gene repression mechanism.

STRUCTURE ELUCIDATION OF ENDOGENOUS LIGANDS OF NUCLEAR HORMONE RECEPTORS AND OTHER SIGNALING MOLECULES THROUGH DIFFERENTIAL ANALYSIS BY 2D NMR SPECTROSCOPY

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Endogenous small molecule ligands of many receptor proteins such as G-protein couples receptors (GPCRs) and nuclear hormone receptors (NHRs) remain unknown due to loss of compound and/or biological activity during successive rounds of activity-guided fractionation of a metabolite extract. However, extensive fractionation aiming to isolate the active component is perceived as mandatory when trying to elucidate the structures of novel or unexpected ligands. Here we present an NMR-spectroscopy-based method - Differential Analysis by 2D NMR Spectroscopy (DANS) - that enables structure elucidation early in the fractionation process, based on spectra of chemically still very complex samples.
By applying this method we were able to identify new structures of signaling molecules active in key pathways that regulate developmental progression of the model organism *C. elegans*. Developmental progression in *C. elegans* is regulated by two classes of signaling molecules: "Dauer pheromones" or "ascarosides" and steroid hormones called "dafachronic acids". Application of DANS facilitated access to the structures of novel ascarosides and dafachronic acids. Synthetic samples of these new endogenous signaling molecules could be used to investigate the signaling cascades they participate in *in vivo*. We show that the synthesized compounds constitute powerful tools when combined with genetics of genes that act upstream, downstream or in parallel to the receptor gene. This work highlights the potential of the DANS analytical method and its potential for structure elucidation of a wide variety of signaling molecules in metazoans.

THE IMMUNOMODULATORY GLYCAN LNFPIII ATTENUATES METABOLIC SYNDROME IN HFD-INDUCED OBESITY

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Alternative activation of the immune system by Th2 cytokines is thought to exert anti-inflammatory activities, which are beneficial in reversing diseases associated with chronic inflammation. Therapeutics designed to skew the immune system to a Th2 profile are less than ideal because of their potential to aggravate autoimmunity and allergies. It remains unclear whether immunomodulatory molecules that display anti-inflammatory phenotypes can be used to treat metabolic syndrome and related inflammatory diseases. Here we show that HFD-induced obese mice treated with the immune modulatory glycan LNFPIII have improved insulin sensitivity, less lipid accumulation in the liver, and reduced inflammation systemically compared to their untreated controls. LNFPIII treatment improves insulin sensitivity in metabolically active tissues by activating insulin-signaling pathways. Furthermore, this signaling event appears to be initiated by macrophage derived cytokines. Taken together, our study suggests that immunomodulatory glycans may serve as a novel therapeutic approach to improve insulin sensitivity in metabolically active tissues and reverse the harmful effects of metabolic syndrome.

STRUCTURAL STUDIES OF TRANSCRIPTIONAL NUCLEAR RECEPTOR COMPLEXES INVOLVING ERR AND PGC-1α

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Transcriptional regulation of metabolic function is intimately dependent on the nuclear receptor family of transcriptional factors and in particular on the estrogen-related receptor ERR. Because ERRs function in a ligand-independent way their transcriptional activity depends on the presence or absence of transcriptional coactivators such as the peroxisome-proliferator-activated-receptor gamma coactivator 1α (PGC-1α), the master regulator of mitochondrial biogenesis and energy metabolism. ERR and PGC-1α are expressed at elevated levels in tissues subjected to high energy demand, such as the heart and brown adipose tissue, and they are co-induced in a tissue-specific fashion during development and in response to physiological stress.
Structurally, most of our current knowledge on the molecular basis of the mechanism of action of nuclear receptors is based on the X-ray and NMR structures of the separated DNA and ligand binding domains. Furthermore, structural information on receptor/coactivator complexes is so far limited to peptides containing LXXLL motifs. Here we address the molecular mechanisms of coactivator recognition by ERR by using a structural approach combining analytical ultracentrifugation, small angle X-ray scattering (SAXS) and other biophysical and biochemical characterization tools. We show that a homodimeric ERRa complexed to its DNA response elements binds PGC-1α with a well defined stoichiometry. The SAXS data provide first molecular envelopes of the coactivator/receptor/DNA complex suggesting the possible localization of PGC-1α on the ERR homodimer bound to the DNA.

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IN VITRO BIOTOOLS FOR NR RESEARCH: FROM WHOLE CELL BIOSENSORS AND –OMICS BASED METHODS TO USE OF PRECISION CUT ORGAN SLICES AND USSING CHAMBERS

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There is a whole battery of in vitro tools to study the mechanism of action of nuclear receptor (NR) ligands. Receptor-reporter gene assays based on mammalian cells are often very sensitive, showing maximal responses to steroids in the pM range. *In vivo*, however, 1 nM 17β-estradiol, the circulating level in premenopausal women, is required to maximally activate the ERα. The sensitivity of many mammalian cell lines is most likely reflecting the selection of these tumour cell lines during competition of growth. It's thus questionable whether these cell lines are the most suited models to study NR activity. Yeast based assays show comparable sensitivities towards natural hormones as normal healthy human cells and do not suffer from crosstalk of other NRs. Combined with models to mimic metabolism and bioavailability they offer great tools to specifically study NR activity.
Here we used bovine liver slices to study both the metabolism and the effect of steroids on gene expression. Liver slices exposed to the AR inactive pro hormone DHEA revealed 1) an increase in androgenic activity as observed by the yeast bioassay, and 2) the formation of 7α-hydroxy-DHEA, 7-oxo-DHEA, 5-androstene-3,17-diol, 17α-testosterone, 4-androstenedione (4-AD), and 17β-testosterone as detected by LC-TOFMS analysis. The same liver slices were subjected to expression microarray analysis. DHEA exerted gene expression changes that were very similar to those induced by 17β-T. Taken together, this demonstrates that DHEA is bioactivated by liver slices, enabling detection of prohormones both by the yeast bioassay and by gene expression.
A similar approach was used for 2-isopropylthioxanthone (ITX), a photoinitiator widely used in printing ink. Due to the absence of sufficient toxicological data, an adequate risk assessment for ITX was lacking. The yeast estrogen and androgen bioassays revealed ITX to exert both anti-estrogenic and anti-androgenic activity. The DR CALUX® bioassay demonstrated ITX to activate the Ah-receptor. The potencies of ITX detected by these bioassays were confirmed by expression microarray studies on rat liver cells exposed either *in vitro* or *in vivo* to ITX and using TCCD, Casodex and Flutamide as reference compounds.
Presently we perform experiments using the Ussing chamber model to study the transport of ITX and it's effect on metabolism and gene expression in rat intestine. These studies show that chemical-analytical, bioassay, organ slices, Ussing Chamber, and –omics-based analysis are complementary, offer the possibility to perform more adequate risk assessments and might eventually be able to replace animal testing.

GLUCOCORTICOIDS ENHANCE THE EXPRESSION AND FUNCTION OF THE NLRP3 INFLAMMASOME

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Glucocorticoids have long been recognized as powerful anti-inflammatory compounds and are, in fact, is one of the most widely prescribed class of drugs in the world. Emerging evidence suggests that glucocorticoids may also have potent pro-inflammatory potential. In light of this, we sought to examine the effects of glucocorticoids on the NOD-like receptors (NLRs). The NLRs form the central component of a multiprotein complex known as the inflammasome and are essential for the activation and release of the pro-inflammatory cytokines IL-1 beta and IL-18. NLRP3, one of the most-widely studied NLRs, is activated in response to a wide variety of pathogen- and danger-associated molecular patterns, including LPS and ATP, respectively. Furthermore, germline and somatic mutations in NLRP3 are the underlying cause of a number of autoimmune diseases, such as Muckle-Wells syndrome and Familial cold autoinflammatory syndrome. NLRP3 has a wide tissue distribution, but is highly expressed in macrophages and osteoblasts. As a model system, the monocytic cell line THP-1 was differentiated into macrophages by phorbol-ester treatment. Following differentiation, THP cells are able to process and secrete IL-1 beta in response to NLR agonists. Interestingly, we have found that the synthetic glucocorticoid, dexamethasone, led to a significant increase of NLRP3 messenger RNA while not significantly changing other components (i.e., caspase-1 or IL-1 beta). Functionally, concomitant administration of dexamethasone significantly enhanced ATP-mediated release of mature IL-1 beta in a dose- and time-dependent manner. This effect is specific for glucocoritcoids, as only cortisol but not aldosterone, estrogen or testosterone was able to enhance secretion of IL-1 beta following ATP stimulation. Furthermore, pretreatment with the glucocorticoid receptor antagonist Ru486, blocked the dexamethasone-dependent increase in IL-1 beta release. Finally, in addition to IL-1 beta, dexamethasone also enhanced the release of the pro-inflammatory cytokine TNF-alpha following ATP stimulation. These results demonstrate a novel role for glucocorticoids in the initial inflammatory response by the innate immune system in response to insult.

LOSS OF IKKξ IMPROVES ENERGY EXPENDITURE, DIET-INDUCED INFLAMMATION AND HEPATIC STEATOSIS INDEPENDENTLY OF BODY WEIGHT

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Obesity is associated with low-grade inflammation that negatively impacts insulin sensitivity. We recently reported that high fat diet (HFD) leads to a sustained increase of IkB kinase ξ (IKKξ) mRNA, protein and activity in liver, adipocytes and adipocyte tissue macrophages. IKKξ knockout mice were surprisingly resistant to diet-induced weight gain, chronic inflammation in liver and white adipose tissue (WAT), hepatic steatosis and whole-body insulin resistance. Hyperinsulinemic-euglycemic clamp studies revealed a temporal change in insulin sensitivity in different tissues. IKKξ knockout produced improved suppression of hepatic glucose production after 2 months of HFD, while improved glucose uptake in muscle was not significant in knockout mice until 4 months of HFD.

To ascertain the contribution of differences in weight gain to the improved metabolic profile of knockout mice, we performed metabolic studies on weight-matched animals after shorter (6-8 weeks) HFD, a point at which hyperglycemia and hyperinsulinemia have just developed. Interestingly, weight-matched IKKξ knockout mice exhibited increased whole body oxygen consumption and increased UCP-1 expression in WAT, independent of obesity. Both liver and serum triglycerides were significantly lower in the weight-matched KO mice. Diet-induced macrophage and T cell infiltration were markedly reduced in both WAT and liver, while the inflammatory cytokine expression was significantly lower only in livers of the KO mice. However, glucose and insulin levels were indistinguishable at this time point.

To identify downstream targets of IKKξ, we performed beadchip analyses comparing gene expression profiles in liver and WAT of WT and weight-matched KO mice. The majority of differences were observed in metabolic genes in both tissues, although inflammatory genes were also lowered in liver of knockout mice. These data suggest that upon its induction after HFD, IKKξ exerts profound direct effects in liver and adipose tissue to regulate energy expenditure and inflammation.

REGULATION OF THE HISTONE DEMETHYLASE LSD1 BY NUCLEAR HORMONE RECEPTORS.

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Nuclear receptors (NRs) regulate a wide range of physiological processes and their action occurring at the level of transcription has been found to involve dynamic regulation of chromatin structure. Histone methylation plays a fundamental role in regulating both chromatin structure and transcription. The histone demethylase LSD1 has been shown to act as both a transcriptional co-repressor with histone H3 di-K4 demethylase activity and a co-activator with H3 di-K9 demethylase activity. We hypothesize that histone modifying enzymes like LSD1 can be regulated by the proteins they interact with. Transcription factors such as NRs are likely candidates as regulators of histone modifying enzymes given their duel roles in transcription activation and repression. Here we show that multiple NR proteins are capable of inhibiting LSD1 H3 di-K4 HDM activity but do not alone convert LSD1 to a H3 di-K9 demethylase. We also propose possible mechanisms for LSD1 ability to contribute to both activation and repression of gene transcription.

PROGESTERONE RECEPTOR ACTIVITY MAINTAINS UTERINE QUIESCENCE THROUGH DIRECT REGULATION OF CASPASE-3 ACTION IN THE PREGNANT UTERUS.

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The appropriate timing of the onset of labor is critical to a successful pregnancy with devastating consequences with the occurrence of pre-term birth. Reduced uterine progesterone receptor (PR) function through administration of the PR antagonist RU-486 to pregnant mice or women induces the onset of pre-term labor at any gestational time point. Whereas administration of exogenous progesterone (P4) can decrease the risk of pre-term birth in high-risk human pregnancies and delay the onset of labor in mice. However the mechanism and targets of P4 and PR action that mediate uterine quiescence are unknown. We have identified a direct target of PR action in the pregnant uterus, uterine caspase-3, that is directly linked to the contractile ability of the uterine myocyte. We hypothesize that non apoptotic uterine caspase-3 action maintains the uterine myocyte in a state of contractile dysfunction through proteolytic cleavage of components of the myocyte contractile architecture utilizing conserved caspase-3 cleavage sites. Although activation of caspase-3 is typically associated with the onset of apoptosis other studies have also identified caspase-3 as a negative regulator of myocyte contractility in cardiac, skeletal and smooth muscle without resulting in cell death.

We have identified four putative P4 response elements (PRE's) in the mouse and one in the human, 2Kb upstream from the pro-caspase 3' start site of transcription. Utilizing a human myometrial cell line, we have identified by ChiP analysis a 7-fold increase in recruitment of the PR mediated by P4 which was ablated in the presence of RU-486. We have found that administration of P4 to pregnant mice delays the onset of labor through upregulation of uterine caspase-3 action resulting in elevated proteolytic cleavage of components of uterine contractile architecture such as smooth muscle α and γ actin. On the other hand administration of 150μg RU-486 on E13 to pregnant mice precipitates the onset of pre-term birth as a consequence of diminished uterine caspase-3 action and precocious reconstitution of the uterine contractile architecture permitting the onset of uterine contractions. Force transduction analysis utilizing pan caspase inhibitors confirm that inhibition of uterine caspase-3 activity results in increased contractile potential. Together these data define caspase-3 as the first protein found in the pregnant uterus that can be directly associated with the contractile potential of the uterus during pregnancy and to be a direct target of increased P4 and PR action.

THE DLK GENE IS A TARGET FOR PPARγ-MEDIATED TRANSCRIPTIONAL ACTIVATION DURING BOTH ADIPOGENESIS AND NEUROGENESIS.

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DLK, a mixed-lineage kinase family member, is a regulator of development, cell differentiation, apoptosis and neuronal response to injury. Recent studies from our laboratory showed interestingly that DLK expression is up-regulated in 3T3-L1 cells induced to differentiate into adipocytes and that DLK depletion by RNA interference impairs the expression of genes known as master regulators of adipogenesis, including the peroxisome proliferator-activated receptor-γ (PPARγ). Because the PPARγ agonist rosiglitazone was found to increase the expression of endogenous DLK in 3T3-L1 cells, we hypothesized that PPARγ is required for the transcriptional activation of the DLK gene. To test this notion, we first examined the effects of pharmacological inhibition or shRNA-mediated depletion of PPARγ in 3T3-L1 cells on DLK protein expression. Besides blocking adipocyte conversion of 3T3-L1 cells, inhibition of PPARγ completely abolished the accumulation of DLK seen upon exposure of cells to the differentiation cocktail. Moreover, mice treated orally with the PPARγ agonist rosiglitazone respond with increased DLK protein level in the mesenteric adipose tissue depot when compared to controls. In support for a role of PPARγ in activating DLK gene transcription, two potential PPARγ binding sites, located at -611 and -769 base pairs upstream of the transcription start site, were identified in the proximal promoter of the DLK gene using bioinformatic tools. Chromatin immunoprecipitation with antibodies against PPARγ revealed that these sites become occupied by PPARγ as differentiation proceeds. The sequence surrounding the potential PPARγ sites, but not a control sequence located 1 kb upstream, was also enriched for RNA polymerase II and retinoid X receptor (RXR), the essential heterodimer partner of PPARγ. In addition, we show that the binding of PPARγ, RNA polymerase II and RXR to the DLK proximal promoter correlates with the increase in DLK protein levels observed during 3T3-L1 preadipocyte differentiation. Taken together, these results show that the DLK gene is a target for PPARγ-mediated transcriptional activation in differentiating 3T3-L1 adipocytes. Interestingly, this mechanism of regulation is not unique to adipocytes because similar results were obtained in Neuro-2a neuroblastoma cells undergoing differentiation. Since PPARγ plays a central role in both adipogenesis and neurogenesis, it is tempting to speculate that DLK, one of its target genes, represents a potential candidate in the etiology of obesity and/or neurodegenerative illnesses.

SELECTIVE MODULATION OF GR CAN DISTINGUISH BETWEEN TRANSREPRESSION OF NF-κB and AP-1

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Glucocorticoids (GCs) can relieve the symptoms of various inflammatory and immune disorders through interferences with the activation and/or activity of pro-inflammatory transcription factors NF-κB and AP-1, a mechanism known as transrepression. However, as GCs can additionally control genes involved in sugar, lipid and protein metabolism, mostly via a mechanism known as transactivation, chronic usage in patients suffering from immune disorders results in a range of undesirable effects. A controlled restriction in the targeted signaling pathways affected by GR is therefore predicted to result in a higher specificity and selectivity towards immune modulation. We previously characterized the action mechanism of a dissociated non-steroidal GR modulator CpdA, which favors GR transrepression over transactivation mechanisms via stimulating monomeric GR formation. Here, we explored how selective modulation of GR affects the activation and activity of AP-1.

Our results demonstrate that CpdA, unlike classic GCs, selectively blocks NF-κB- but not AP-1-driven gene expression. CpdA rather sustains AP-1-driven gene expression, a result which could be mechanistically explained by the failure of CpdA to block upstream activated JNK kinase and concomitantly also phosphorylation of c-Jun. In concordance, CpdA sustained the expression of the activated AP-1 target gene c-jun, as well as the production of the c-Jun protein. Finally, ChIP analysis data clearly demonstrates that only DEX-activated GR, but not CpdA-activated GR, is recruited to AP-1-driven promoters. The molecular basis of this selectivity is currently being explored, but already fits with the assumption that a ligand-induced differential conformation of GR may expose different interaction surfaces and thus yield a different transcription factor interaction profile.

KDB and IMB are postdoctoral fellows at the Research Foundation - Flanders (FWO-Vlaanderen).
* KDB and IMB contributed equally

INCREASED GLUCOCORTICOID RECEPTOR EXPRESSION AND ACTIVITY MEDIATE THE LPS RESISTANCE OF SPRET/EI MICE.

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SPRET/Ei mice are extremely resistant to acute LPS-induced lethal inflammation when compared to C57BL/6. We found that in vivo SPRET/Ei mice exhibit strongly reduced expression levels of cytokines and chemokines. To investigate the role of the potent anti-inflammatory glucocorticoid receptor (GR) in the SPRET/Ei phenotype, mice were treated with the GR antagonist RU486 or bilateral adrenalectomy. Under such conditions, both C57BL/6 and SPRET/Ei mice were strongly sensitized to LPS and the differences in LPS response between SPRET/Ei and C57BL/6 mice were completely gone. These results underscore the central role of GR in the LPS hyporesponsiveness of SPRET/Ei mice. Compared to C57BL/6, SPRET/Ei mice were found to express higher GR levels, which were reflected in increased GR transactivation. Using a backcross mapping strategy, we demonstrate that the high GR transcription levels are linked to the Nr3c1 (GR) locus on chromosome 18 itself. Unexpectedly, SPRET/Ei mice exhibit a basal overactivation of the hypothalamic-pituitary-adrenal axis, namely strongly increased corticosterone levels, ACTH levels and adrenocortical size. As a consequence of the excess of circulating glucocorticoids (GCs), levels of hepatic gluconeogenic enzymes are increased and insulin secretion from pancreatic β-cells is impaired, both of which result in hyperglycemia and glucose intolerance in SPRET/Ei mice. We conclude that SPRET/Ei mice are unique since they display an unusual combination of elevated GR expression and increased endogenous GC levels. Hence, these mice provide a new and powerful tool for the study of GR- and GC-mediated mechanisms, including immune repressive functions, neuroendocrine regulation, insulin secretion, and carbohydrate metabolism.

GRIP1 AS A TARGET FOR GLUCOCORTICOID-INDUCED PHOSPHORYLATION

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The widespread use of glucocorticoids (GC) as highly potent immunosuppressors is hampered by pleiotropic side effects on metabolism necessitating a better mechanistic understanding of the GC actions. Glucocorticoid receptor (GR), which conveys the biological activities of GCs, regulates transcription of a wide variety of genes by recruiting distinct multiprotein co-regulator complexes in a gene- a response element-specific manner. A p160 family member, TIF2/GRIP1 is one of the key cofactors facilitating both GR-mediated activation and repression by serving as a platform for the assembly of histone modifying and chromatin remodeling complexes. Interestingly, GRIP1 also serves as a coactivator for the key effectors of toll-like receptor (TLR) and type I interferon (IFN) signaling, interferon regulatory factors (IRF)3 and 9, respectively, enhancing the transcription of innate immune response genes. GR activation triggers loss of GRIP1 from IRFs, which contributes to GC-induced immunosuppression. The exact mechanisms facilitating GRIP1 recruitment to specific genes and regulatory complexes, or those preferentially 'turning on' its coactivator or corepressor properties remain to be elucidated.
Unexpectedly, we found that GRIP1 is phosphorylated in response to GC stimulation. Indeed, upon exposure of cultured cells to a synthetic GC, dexamethasone (Dex), GRIP1 protein undergoes a dramatic and transient electrophoretic mobility shift, which is abolished by the treatment of cell lysates with calf intestinal phosphatase. GC-induced GRIP1 phosphorylation was dependent on a physical interaction between GRIP1 and GR, as disruption of the GR "charge clamp" or GRIP1 NR box-3, required for the GR:GRIP1 interface formation, abolished GRIP1 mobility shift. Likewise, treatment with partial GR antagonist RU486 which induces GR conformation incompatible with GRIP1 binding, eradicated the GRIP1 shift in a dose-dependent manner. These data indicate that GR likely brings a protein kinase into the complex to modulate the activity of its own cofactor via phosphorylation. Based on the results of a pilot screen for the GRIP1 kinases using a panel of inhibitors, we are testing several candidate kinases for their ability to phosphorylate GRIP1 in vitro. We have identified by mass spectrometry a series of endogenous phosphorylation sites (6 hormone-dependent and one constitutive) whose functional significance is presently being characterized. We propose that GR-dependent GRIP1 phosphorylation influences transcription of GR target genes and may dictate the preferential use of GRIP1 by GR over other transcriptional regulatory complexes such as those triggered by the TLR or IFN signaling.

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NUR77-DEPENDENT DEREGULATION OF PPARγ2 IN FASTED WHITE ADIPOSE TISSUE

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Expression of the nuclear receptor Nur77 (NR4A1) is induced in WAT in response to fasting conditions and β-adrenergic stimulation. We therefore performed microarray-based expression profiling of WAT from wildtype and Nur77$^{-/-}$ mice submitted to prolonged fasting. Results revealed Nur77-dependent changes in expression profiles of 153 transcripts ($P \leq 0.05$). Mapping those transcripts onto functional associations and known biochemical pathways revealed connections to insulin signalling, and to lipid, fatty acid and glucose metabolism. Additionally, Nur77-dependent expression profiles were assayed in mature 3T3-L1 adipocytes with and without Tet-induced Nur77 overexpression, submitted to β-adrenergic stimulation. As several of the Nur77-regulated genes are known PPARγ2 targets (ie. G0s2, Grp81, Fabp4, and Adiponectin) we further assayed the expression level of PPARγ2 in a bigger cohort of fasted mice and found a significant Nur77-dependent regulation of PPARγ2 (P=0,021, n=10). Furhermore, we show with chromatin immunoprecipitation and luciferase assays that the PPARγ2 promoter is a direct target of Nur77-dependent regulation. In conclusion, we present data implicating Nur77 as a regulator of PPARγ2 in WAT.

THE ROLE ERK1/2 AND ANDROGEN RECEPTOR PHOSPHORYLATION AT SERINE 81 IN THE PROGRESSION TO CASTRATE RESISTANT PROSTATE CANCER.

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Prostate cancer incidence in the UK is approximately 35,000 cases per year and is the second most common cause of male cancer-specific death. Treatment of choice for locally advanced or metastatic disease is maximum androgen blockade. The majority of men that receive this therapy eventually relapse with castrate resistance prostate cancer (CRPC).Cell line studies implement ERK1/2 activition in the transition from hormone naïve prostate cancer (HNPC) to CRPC. Evidence suggests that ERK kinase activation induces phosphorylation of AR at Serine 81to stimulate prostate cancer cell growth and induce PSA expression.
The aim of the current study was to investigate if the relationship between the AR and ERK1/2 is upheld in clinical prostate cancer specimens. A cohort of 55 patients with matched HNPC and CRPC tissue was established with full clinical follow-up. Immunohistochemistry was employed to assess expression of AR, AR81, ERK1/2 and phosphorylated ERK1/2 (pERK).
All 55 patients in the cohort were diagnosed with HNPC and subsequently progressed to CRPC.In the transition for HNPC to CRPC 8% of patients exhibited an increase in AR81 expression and 20% exhibited an increase in ERK1/2 expression.PSA at diagnosis (p=0.034) and high pERK expression in HNPC (p=0.03) were associated with shorter time to biochemical relapse. A negative correlation between AR and ERK1/2 expression was observed in HNPC (R2=-0.383, p=0.004), but no association with AR81 and ERK1/2 or pERK was observed in this stage of disease.
Gleason sum (p=0.009), presence of metastasis at diagnosis (p=0.0002), presence of metastasis at relapse (p=0.003) and high ERK1/2 expression in CRPC (p=0.003) was associated with shorter disease specific survival. In CRPC no correlations were observed between AR, AR81 and ERK1/2 or pERK. AR81 expression did however positive correlated with proliferation index as assessed by Ki67 expression (R2=0.339, p=0.025). When expression of AR81 and ERK1/2 were combined, disease specific survival was significantly shorter when both were expressed compared expression of one or expression of neither (4.2 years vrs 5.4 year vrs 7.6 years respectively, p=0.0006).
No correlations with AR81 and ERK1/2 or pERK were observed,high expression of AR81 and ERK1/2 combined had a synergistic effect of decreased disease specific survival. Interactions between both these proteins warrant further investigation.

GENOME-WIDE EFFECTS OF RNAI-MEDIATED KNOCKDOWN OF STEROIDOGENIC FACTOR-1 IN ADRENOCORTICAL CELLS

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Steroidogenic factor-1 (SF-1, NR5A1) is a nuclear receptor (NR) primarily involved in the development and homeostasis of the steroidogenic organs in the hypothalamic-pituitary-adrenal/gonadal axis. It governs the expression of enzymes responsible for converting cholesterol to steroid hormones, including the mitochondrial cholesterol transport protein StAR and members of the cytochrome P450 family of enzymes. The structure of the SF-1 ligand-binding domain revealed phospholipids in the ligand binding pocket and reports of synthetic ligands affecting SF-1 transcriptional activity have started to emerge. In vivo regulation of SF-1 is known to include posttranslational modifications where phosphorylation can activate and SUMOylation repress its transcriptional activity. SF-1 repression also occurs via interactions with a corepressor complex containing the NR DAX-1 and, as we have shown (Mol Cell Biol 29: 2230-42, 2009), the ubiquitin ligase RNF31, HDAC3 and SMRT.

It was recently shown that SF-1 is over-expressed in childhood adrenocortical tumors and that SF-1 can increase proliferation of adrenocortical cells, expanding the role of SF-1 beyond steroidogenic regulation. In light of this, we decided to take a genome-wide approach to investigate the role of SF-1 in the adrenal cortex by using RNAi-mediated knockdown in the adrenocortical carcinoma cell line H295R followed by microarray analysis. We find that SF-1 is important for controlling pathways involved in both steroidogeneseis and cell proliferation. New possible targets of SF-1 regulation are identified and old ones confirmed. We also assess the effect of cAMP-treatment when cells are depleted of SF-1, giving an indication of the role of SF-1 in basal versus induced steroidogenesis. We hope that our data will help advance the understanding of the multifaceted roles of this important receptor.

PGC1A-INDEPENDENT REGULATION OF VEGF AND VASCULARIZATION BY ERRγ IN SKELETAL MUSCLE

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Type I aerobic muscle inherently maintains high oxidative and vascular capacity even without exercise. How this occurs could have important implications in treatment of vascular disease and ischemia when exercise may not be possible. Here we show that the orphan nuclear receptor ERRγ, a potent metabolic and mitochondrial regulator, is expressed at high levels in type I muscle but not in type II glycolytic muscle. However, transgenic expression of ERRγ in glycolytic type II muscles (ERRGO mice) or cultured cells, powerfully regulates VEGF expression, angiogenesis and vascular supply in absence of exercise. ERRGO mice show increased expression of genes promoting fat metabolism, mitochondrial respiration and type I fiber specification. In parallel, the type II muscle in ERRGO mice display an activated angiogenic program marked by myofibrillar induction and secretion of pro-angiogenic factors, frank neo-vascularization and a 100% increase in running endurance. Surprisingly, the induction of VEGF and type I muscle properties by ERRγ does not involve the transcriptional co-activator PGC1α or Hif1α which remain at basal levels. Instead, ERRγ “genetically activates” the energy sensor AMPK which is typically inactive in absence of exercise. Therefore, ERRγ and AMPK, known regulators of mitochondrial function and metabolism, together control a novel angiogenic pathway that anatomically synchronizes vascular arborization to oxidative metabolism revealing an exercise-independent mechanism for matching supply and demand.

FOXO1 REGULATES TLR4 INFLAMMATORY PATHWAY SIGNALING IN MACROPHAGES

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The macrophage-mediated inflammatory response is a key etiologic component of obesity related tissue inflammation and insulin resistance. The transcriptional factor FoxO1 is a key regulator of cell metabolism, cell cycle and cell death. Its activity is tightly regulated by the phosphoinositide-3-kinase-AKT (PI3K-Akt) pathway, which leads to phosphorylation, cytoplasmic retention and inactivation of FoxO1. Here, we show that FoxO1 promotes inflammation by enhancing Tlr4-mediated signaling in mature macrophages. By means of chromatin-immunoprecipitation combined with massively parallel sequencing (ChIP-Seq), we show that FoxO1 binds to multiple enhancer-like elements within the Tlr4 gene itself, as well as to sites in a number of Tlr4 signaling pathway genes. While FoxO1 potentiates Tlr4 signaling, activation of the latter induces AKT and subsequently inactivates FoxO1, establishing a self-limiting mechanism of inflammation. Given the central role of macrophage-Tlr4 in transducing extrinsic proinflammatory signals, the novel functions for FoxO1 in macrophages as a transcriptional regulator of the Tlr4 gene and its inflammatory pathway, highlights FoxO1 as a key molecular adaptor integrating inflammatory responses in the context of obesity and insulin resistance.

USP7/HAUSP STABILIZES TIP60 IN ADIPOGENESIS

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Posttranslational modifications (PTMs) present an important mechanism to regulate the activity of transcriptional coregulators. While de-modification may play an extremely important role in balancing the transcriptional output, no enzymes have been identified so far which can for example de-ubiquitinate coregulator proteins. We have previously shown the acetyltransferase Tip60 to be essential for 3T3-L1 adipogenesis. Interestingly, Tip60 protein but not mRNA expression increased during differentiation, suggesting that Tip60 protein levels may be regulated by ubiquitination-deubiquitination. Here we show that the deubiquitinase USP7/HAUSP stabilizes Tip60 in adipogenesis. Using a probe which only binds to active deubiquitinases (DUBs), we found only 2 DUBs to be activated during 3T3-L1 adipogenesis, USP7/HAUSP and UCHL3. These two proteins also present the major DUB activities in mouse white and brown adipose tissue. In common with disruption of Tip60 function, siRNA-mediated reduction of USP7 protein impairs differentiation of 3T3-L1 pre-adipocytes. The Tip60 and USP7 proteins are both located in the nucleus of differentiated 3T3-L1 cells, and the two proteins interact in these cells. Co-expression of USP7 with Tip60 leads to the stabilization of Tip60 and increases its steady-state level, and Tip60 is deubiquitinated by USP7 both *in vivo* and *in vitro*. Knockdown of USP7 in 3T3-L1 adipocytes leads to the down regulation of Tip60 protein level, underscoring the role of USP7 in the stabilization of Tip60 in adipocytes. Since both USP7 and Tip60 are important regulators of gene expression and chromatin structure and are both important in p53 pathway, this interaction could have more general implications in cell differentiation and metabolism.

CACTIN, A NOVEL REGULATOR OF TLR SIGNALLING PATHWAYS

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Toll-like receptors (TLRs) recognise specific microbial motifs and respond by inducing pro-inflammatory genes that govern both innate and adaptive immune responses. Activation of TLRs initiates a signalling cascade which leads to the induction of transcription factors such as NF-κB and interferon-regulatory factors (IRFs) which regulate gene expression. Dysregulation of these signalling pathways can lead to inflammatory diseases and so these pathways are tightly controlled by negative regulators of immune signalling. In 2000, Lin *et al.* indentified Cactin (*Cact*us *in*teractor) as a novel interactor of *Drosophila* Cactus, a regulator of *Drosophila* Toll signalling. We have cloned the human ortholog of Cactin and show that it acts as a negative regulator of TLRs. Overexpression of hCactin suppresses TLR-induced activation of NF-κB and IRF transcription factors whereas knockdown of hCactin augments the induction of these responses. hCactin localises to the nucleus and this nuclear localisation is critical for manifesting its inhibitory effects on TLR signalling. Cactin does not interact with the human ortholog of Cactus (IκBα) but does interact with nuclear IκB-like and other proteins that are resident of the nucleus. These findings define Cactin as a novel negative regulator of TLR signalling that exhibits its regulatory effects in the nucleus.

EXPRESSION PROFILING DEFINES A UNIVERSAL GLUCOCORTICOID RESPONSE PROGRAM IN AIRWAY SMOOTH MUSCLE, INCLUDING NON-INFLAMMATORY TARGETS THAT ALTER AIRWAY FUNCTION.

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The lung is one of the key client tissues for glucocorticoids in the clinic, however, a detailed understanding of the target genes that are controlled by the glucocorticoid receptor (GR) in various lung cell types, nor their physiologic impact, does not exist. These knowledge gaps are barriers to both the development and evaluation of novel therapies for lung disease that modulate GR signaling. To address this issue, we used expression profiling, high throughput qPCR, and gene ontology analysis to annotate the GR-regulated transcriptome in cultured airway smooth muscle (ASM), a critical target cell type for asthma therapeutics. Several lines of investigation emerged from this initial discovery process. First, we performed detailed qPCR validation for a subset of 40 of the 487 genes identified with a high degree of confidence by microarray (adjusted $p < .01$) as regulated by GR after 4 hours of treatment with dexamethasone. We found that this gene subset exhibited a very high degree of concordance with respect to GR regulation in 6 independent primary human ASM lines derived from different donors. Second, we found that activation of GR signaling in ASM rapidly reduces mRNA levels of numerous genes in a manner consistent with GR regulating mRNA stability; again this observation was consistent across numerous ASM lines. Third, to establish the *in vivo* impact of non-inflammatory GR-regulated genes in ASM, we analyzed airway contractility in mice lacking Klf15, a GR target gene that had no known function in the lung. We found reduced airway contraction in Klf15$^{-/-}$ mice induced to develop asthma through allergy sensitization followed by exposure to aerosolized allergen. Intriguingly, inflammation was indistinguishable between Klf15$^{-/-}$ and wild type mice, suggesting that Klf15 impacts airway contractility in a manner that does not depend on a reduction in the allergic response. Together, our results indicate that: 1) the GR response is surprisingly similar across primary cultured airway cell lines derived independently from different donors; and 2) non-inflammatory GR target genes in the lung potentially contribute to the therapeutic impact of GR activation and thus might be therapeutic targets in their own right. We propose that annotation of the GR transcriptome in primary cells or tissues followed by selected *in vitro* and *in vivo* target validation is a useful experimental paradigm to define mechanisms of therapeutic effect and new drug targets in clinically important GR client tissues.

GENOME-WIDE BINDING OF ESTROGEN RECEPTOR A IN RESPONSE TO BISPHENOL A, GENISTEIN OR ESTRADIOL EXPOSURE IS CELL-TYPE SPECIFIC AND ASSOCIATES WITH FOXA1 BINDING

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Estrogen signaling plays important and diverse roles in many tissues, stimulating some tissues to grow while keeping other tissues quiescent. We are exposed to estrogenic compounds such as genistein (GEN), which is made in plants, and bisphenol A (BPA), which is synthesized industrially. To understand the molecular consequences of exposure to environmental estrogens in different cell types, we induced ECC-1, a human endometrial cancer line, and T-47D, a human breast cancer line, with BPA, GEN, and the endogenous estrogen estradiol (E2). We then performed ChIP-seq to measure estrogen receptor α (ERα) binding across the genome and RNA-seq to analyze gene expression changes. In both cell lines, we observed that 10 nM E2 induced ERα binding at ~8,500 sites across the genome, while 100 nM GEN induced ~5,000 binding sites, and 100 nM BPA induced ~2,000 binding sites. Within a cell line, more than 95% of binding sites induced by GEN and BPA were also induced by E2. RNA-seq data showed that the number of genes that change upon induction with each ligand correlates with the number of ERα binding sites induced by each ligand. Our results indicate that GEN and BPA do in fact induce ERα binding in the genome, although they are weaker agonists of ERα than E2 in both cell lines.

We also found that binding of ERα and mRNA changes induced by E2 treatment were very different between ECC-1 and T-47D. While both cell lines exhibit 8,500 ERα binding sites, we found that only 17% overlap. We analyzed binding sites that were cell line-specific and discovered that T-47D-specific binding sites were enriched in binding sequences for FOXA1, a known ERα interacting transcription factor (Krum SA et al 2008; Carroll JS et al 2005). FOXA1 expression is 100-fold higher in T-47D and therefore served as a good candidate for explaining cell type-specific binding. We performed ChIP-seq of FOXA1 in both lines and discovered more than 30,000 binding sites in T-47D and only 2,500 binding sites in ECC-1, corresponding to mRNA levels of FOXA1. In T-47D there is strong overlap between FOXA1 and ERα binding sites (53% overlap), while in ECC-1 there is modest overlap (18% overlap). This co-occupancy suggests a role for FOXA1 in determining ERα binding sites in T-47D, but not in ECC-1. Overall, these results indicate that different ligands for ERα induce similar binding sites within a cell type, while differences in the interacting factors present in cell types can contribute to very different ERα binding regimes.

JMJD3 REGULATES *BCL-2* TRANSCRIPTION IN ERα-DEPENDENT BREAST CANCER CELLS.

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The steroid hormone estrogen (E2) and its intracellular receptors, the estrogen receptors α (ERα), are integral parts of many signal transduction processes that, if altered, result in diseases such as breast cancer. ERα is known to stimulate transcription of RNA polymerase II target genes by promoting local changes in hormone-responsive promoters embedded in chromatin. In particular, chromatin can be modified by the di- and tri-methylation of histone H3 lysine 27 (H3K27) that has a repressive effect on gene regulation, but can be reversed by the recently identified demethylase, JMJD3, resulting in gene activation. Here we show that JMJD3 and the methylation status of H3K27 are essential in regulation of the ERα-dependent apoptotic response via Bcl-2 in breast cancer cells. The activation of *Bcl-2* transcription is dependent on the demethylation of H3K27 by the E2-dependent recruitment of ERα and JMJD3 in hormone-dependent breast cancer cells. This pathway is modified in cells resistant to anti-estrogens (AE), cells that also constitutively express *Bcl-2*. We show that the promoter structure and transcription status of *Bcl-2* is active in these AE-resistant cells due to a lack of H3K27 methylation. The decrease in H3K27 methylation occurs because overexpressed human epidermal growth factor receptor 2 (HER2), via the kinase AKT, phosphorylates, and thus inactivates the H3K27 methyltransferase EZH2. Inactivation of this pathway via an AKT inhibitor renders these cells sensitive to the estrogen-sensitive regulation of *Bcl-2* dependent on JMJD3. Our results describe a novel mechanism in which the epigenetic state of chromatin affects the ability of a cancerous cell to respond to therapy.

RBCK1 REGULATES PROLIFERATION IN BREAST CANCER CELLS POTENTIALLY THROUGH CONTROL OF ERα GENE EXPRESSION

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Estrogen receptor α (ERα) is a member of the nuclear receptor superfamily and plays a central role in mammary epithelial cell proliferation and growth of estrogen-dependent breast cancer. ERα expression and activity is controlled by estrogen hormones. Upon binding to estrogens, ERα stimulates the expression of genes encoding cell cycle regulatory proteins thereby promoting cellular proliferation. While a major research focus has been directed towards understanding how ERα regulates the expression of its target genes, relatively little is known about the regulation of the ERα gene itself.
Our recent findings identify a role for the E3 ubiquitin ligase RBCK1 in ERα-positive breast cancer cells in supporting cell cycle progression, potentially by driving transcription of ERα. siRNA silencing of RBCK1 led to reduced levels of ERα mRNA and protein levels, reduced ERα target gene expression and reduced estrogen-stimulated entry into the S phase. Chromatin immunoprecipitation (ChIP) revealed that RBCK1 is recruited to the ERα promoter, supporting that transcriptional regulation is one mechanism by which RBCK1 affects ERα mRNA and protein levels. In support of the RBCK1 dependent reduction of ERα mRNA levels in breast cancer cell lines, analysis of breast tumor tissues identified a positive correlation between levels of RBCK1 and ERα mRNA. Considering the critical role of ERα in proliferation of breast cancer, our findings aid in further understanding how to modulate the expression of the receptor, which may ultimately lead to new estrogen-based therapeutic strategies. In the poster, we will present our recent efforts trying to elucidate the functions of RBCK1 critical for its function as a regulator of ERα.

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PLASMA CHOLESTEROL LOWERING BY FXR AGONIST PX20350 IS LIKELY DUE TO INCREASED EXPORT AS WELL AS DECREASED UPTAKE OF CHOLESTEROL

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The Farnesoid X Receptor (FXR, NR1H4) has been shown to be involved in the control of bile acid, triglyceride, and cholesterol homeostasis suggesting that FXR agonists have a potential as new therapeutics in lipid-related disorders. Here we present data obtained with the novel potent FXR agonist Px20350 in order to elucidate possible molecular mechanisms for FXR mediated cholesterol lowering in rodent animal models.
C57Bl6 mice were kept on a 60% kcal high fat/0,5% cholesterol diet (HFCD) and dosed with 10 and 30mpk/d Px20350 for 6 weeks. Px20350 Treatment resulted in a remarkable lowering of both plasma and liver cholesterol and triglyceride levels. Plasma lipoprotein profiling demonstrated a striking reduction of VLDL, LDL and HDL in FXR agonist treated mice. Several genes involved in triglyceride and cholesterol metabolism and transport were significantly down- or upregulated by Px20350. The gene expression data suggested that the export pathways for cholesterol and phospholipids from the plasma (periphery) via the liver into the intestinal lumen were massively increased upon activation of FXR. Furthermore, radiolipid uptake studies in SD rats and C57 mice indicated a reduced uptake of cholesterol and triglycerides by the intestine. The strong reduction of plasma and liver lipids (cholesterol and triglycerides) upon administration of Px20350 is mirrored by a significant reduction of liver fat accumulation which occurs in the control animals under HFCD.
If translatable to humans, the concerted regulation in liver and intestine as well as the magnitude of the lipid lowering effects exerted by FXR agonists open a great therapeutic potential in diseases ranging from atherosclerosis up to Metabolic Syndrome and NAFLD/NASH.

POTENTIATION OF GLUCOCORTICOID SIGNALING BY CHLOROQUINE VIA INHIBITION OF LYSOSOMES.

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Anti-malaria drug chloroquine has been used as an anti-inflammatory agent for treating systemic lupus erythematosus and rheumatoid arthritis, without clear mechanism of action. Here we report that chloroquine potently inhibits the expression of proinflammatory cytokines partially through transrepression of glucocorticoid receptor (GR). Instead of direct binding to GR, chloroquine synergistically activates glucocorticoid signaling via inhibition of lysosomal functions. In mouse collagen induced arthritis model, chloroquine synergizes the therapeutic effects of glucocorticoid. Lysosomal inhibition by bafilomycin A1, an inhibitor of V-type ATPase, or by knockdown of transcription factor EB (TFEB), a master activator of lysosomal biogenesis, mimics the effects of chloroquine. These results reveal an unexpected regulation of glucocorticoid signaling by lysosomes and provide a mechanistic basis for treating inflammation and autoimmune diseases by combination of glucocorticoids and lysosomal inhibitors.

ORPHAN RECEPTOR NR2F6 REPRESSES AUTOIMMUNITY

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PKC signaling to NFAT/AP-1 critically involves the recently identified orphan receptor NR2F6 which is predominantly expressed in the CD4+ T helper cell (Th)17 subset (Hermann-Kleiter et al., Immunity 2008). Mechanistically, PKC-mediated phosphorylation on Ser 83 within the DBD-domain of NR2F6 results in the release of NR2F6 from its DNA binding sites within the *Il17a* promoter, as revealed by EMSA and ChIP analysis. NR2F6 potently antagonizes the ability of Th17 CD4+ T cells to induce expression of key cytokines such as IL-17, IL-23, and IL-21.
In Th17 cells differentiated and activated ex vivo, loss of Nr2f6 results in amplified NFAT DNA binding at the *Il17a* promoter and subsequently increased IL-17 transcription. Consistently, Nr2f6-deficient mice have hyper-reactive lymphocytes and develop late-onset immune-pathologies and are hypersusceptible to the Th17-dependent model of experimental autoimmune encephalomyelitis. Taken together, our study establishes NR2F6 as PKC substrate and critical transcriptional repressor of autoimmunity.

HER2-MEDIATED DOWN-REGULATION OF SMRT IN TAMOXIFEN-RESISTANT BREAST CANCER

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The antiestrogen tamoxifen is the major adjuvant therapy for estrogen receptor alpha (ERalpha)-positive breast cancers. Tamoxifen has successfully decreased the mortality rate of breast cancer patients in the past decades; however, a significant fraction of patients is refractory or acquires resistance to this treatment. Tamoxifen-bound ERalpha recruits transcriptional corepressors SMRT and N-CorR, thereby repressing ERalpha target genes and inhibiting cell cycle progression. We hypothesize that mis-regulation of the corepressors SMRT or N-CoR contributes to tamoxifen resistance. Indeed, several reports including ours suggest that low levels of the corepressors SMRT and N-CoR compromise tamoxifen-mediated repression of ERalpha target genes and its ability to inhibit cell proliferation.

Patients with ERalpha- and Her2/ErbB2-positive are resistant to tamoxifen therapy. Her2/ErbB2, a member of the ErbB transmembrane receptor tyrosine kinase and a potent mitogenic activator, promotes its activity through several downstream effectors. We have previously demonstrated that Her2/ErbB2 down-regulates steady-state levels of SMRT through Cdk2/Pin1-mediated degradation which relieves tamoxifen-mediated repression of ERalpha target genes, and thus renders tamoxifen resistance. In a search for E3 ligases that promote SMRT degradation, we found that the Her2/ErbB2 downstream E3 ligase, β-TrCP1 targets SMRT for degradation in a manner that is independent of Cdk2 or Pin1. SiRNA knockdown and overexpression experiments show that β-TrCP1, but not β-TrCP2 specifically down-regulates SMRT protein accumulation. Protein-protein interaction assays have been used to map the SMRT-β-TrCP1 interaction domains. Based on these observations, we propose a model in which Her2/ErbB2 promotes tamoxifen resistance through Cdk2-Pin1- and β-TrCP1-mediated down-regulation of SMRT protein levels in ERalpha-positive breast cancer patients.

Our future goal is to apply the lessons learned from cell studies and test our hypothesis in xenograft nude mice. Our study will elucidate a novel regulation of SMRT protein homeostasis and provide a new strategy to develop therapeutic agents to breast cancer.

GENOME WIDE ANALYSIS OF FACTORS AFFECTING ANDROGEN RECEPTOR FUNCTION REVEALS NEW THERAPEUTIC TARGETS IN PROSTATE CANCER

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The androgen receptor (AR) is a transcription factor that works by binding androgens to turn genes on or off. The same AR that regulates normal growth and differentiation during male development also plays an integral role in the pathogenesis of prostate cancer. Thus, the cell context in which AR functions, is an important determinant of its actions and consequently, AR-driven prostate cancer. We hypothesize that AR function is controlled by cellular proteins that instruct AR to control gene expression, and that these proteins work differently in cancer cells compared to normal non-growing cells. Thus, our objective is to identify cellular factors that control AR activity as potentially new therapeutic targets for prostate cancer.

Using a high throughput genome-wide RNA interference (RNAi) screen with an AR-responsive reporter gene as the readout, we identified proteins that increase or decrease the activity of AR, thereby generating a comprehensive list of the factors capable of affecting AR actions.

Of the ~14,000 factors screened by RNAi, we identified ~350 candidates, whose depletion from the cell either decreases or increases AR activity. Of those, ~40 selectively affected AR but not glucocorticoid receptor (GR) transcriptional activity. Reassuringly, we isolated factors previously shown to regulate AR function, thus validating the screen. Importantly, the screen yielded new factors that lower AR transcriptional activity including "drugable" targets such as protein kinases. For example, we found that depletion of the homeodomain interacting protein kinase 2 (HIPK2), a nuclear kinase that has been recently shown to participate in transcriptional events, attenuates AR but not GR activity, which could represent new therapeutic target in prostate cancer. In contrast, factors whose RNAi-mediated destruction increases AR activity may represent new tumor suppressor genes.

This is the first genome-wide unbiased screen for mediators of AR function. Knowing what proteins affect AR activity will help in the design of new therapies to treat prostate cancer, especially those refractory to current hormone ablation therapy.

TRANSCRIPTIONAL MEDIATOR SUBUNIT MED1 IN STROMAL CELLS IS INVOLVED IN HEMATOPOIETIC STEM/PROGENITOR CELL SUPPORT THROUGH VDR- AND RUNX2-MEDIATED OSTEOPONTIN EXPRESSION

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The TRAP/Mediator, subcomplex of RNA polymerase II holoenzyme, acts as the end-point integrator of a variety of activators and conveys intracellular signals to the general transcription machinery. Among the subunits, MED1/TRAP220 is crucial for various biological events through its interaction with distinct activators such as nuclear receptors. In hematopoiesis, MED1 plays a pivotal role in optimal VDR- and RAR-mediated myelomonopoiesis and GATA-1-induced erythropoiesis. In this study, we present evidence that MED1 in stromal cells is involved in supporting hematopoietic stem and/or progenitor cells (HSPCs) through osteopontin (OPN) expression. When normal bone marrow (BM) cells were cocultured with mitomycin C-treated *Med1*$^{-/-}$ or *Med1*$^{+/+}$ MEFs, growth of BM cells were significantly suppressed on *Med1*$^{-/-}$ MEFs compared with the control during the two-week period, but recovered when cocultured on *Med1*$^{-/-}$ MEFs into which MED1 was re-introduced (Rev-*Med1*$^{-/-}$ MEFs). Further, the number of long-term culture-initiating cells (LTC-ICs) was attenuated for BM cells cocultured on *Med1*$^{-/-}$ MEFs compared to the control, but recovered when cocultured on Rev-*Med1*$^{-/-}$ MEFs. A microarray analysis of mRNA comparing *Med1*$^{+/+}$ and *Med1*$^{-/-}$ MEFs disclosed approximately 15 genes whose expressions were profoundly attenuated in *Med1*$^{-/-}$ MEFs. Among molecules encoded by these genes we focused on OPN because solely OPN was known to have BM niche function. The *Opn* mRNA was attenuated in *Med1*$^{-/-}$ but recovered in Rev-*Med1*$^{-/-}$ MEFs. Addition of recombinant OPN to *Med1*$^{-/-}$ MEFs restored the growth of cocultured BM cells and the number of LTC-ICs, both of which were attenuated by the addition of the anti-OPN antibody to both *Med1*$^{+/+}$ MEFs and BM stromal cells, OP-9 and MS-5. Luciferase reporter assays disclosed that the basal level, as well as VDR- and Runx2-mediated synergistic activation, of *Opn* transcription was specifically attenuated in *Med1*$^{-/-}$ MEFs, and that the basal transcription and ligand-dependent activation were dependent on the N-terminal domain (amino acids 1 to 602) and NR-recognition motifs of MED1, respectively. ChIP assays disclosed recruitment of Mediator, VDR and Runx2 onto the *Opn* promoter. Taken together, these data suggest that MED1 in niche cells, through upregulating VDR- and Runx2-mediated transcription on the *Opn* promoter, plays an important role in HSPCs support.

DEVELOPMENT OF A NOVEL PHARMACOKINETIC APPROACH FOR THE USE OF GLUCOCORTICOIDS IN THE TREATMENT OF INFLAMMATORY DISEASES

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Background- Glucocorticoids (Gcs) are the most potent anti-inflammatory agents known and are used to treat a number of chronic inflammatory diseases such as inflammatory bowel disease and rheumatoid arthritis (RA). However, their use is limited by side-effects. Therefore, better understanding how Gcs work is of central importance. Gcs are released in pulses from the adrenal gland giving rise to oscillations in blood plasma concentrations. The biological significance of such oscillations has not been explored.
Hypothesis- Pulsatile vs continuous glucocorticoid delivery differentially influences target cell responses.
Meth- A flow-through culture system was established to explore the role of Gc, (hydrocortisone (Hc)) oscillations in HeLa cells and primary T cells. Cells were subjected to either continuous (100ng/ml) or pulsatile (100ng/ml) Hc for 12 hours in the flow-through system. A microarray was performed to identify genes differentially regulated by Hc delivered in pulses compared with continuous treatment in HeLa cells. The expression of 5 such genes (FKBP5, GILZ, GLUL, IL6ST and IL-8) were then analysed in response to pulsatile and continuous Hc treatment by qRT-PCR. Apoptosis was also measured by Annexin V assay. Data were compared using independent sample t test.
Results- Hc increased the expression of endogenous FKBP5, GILZ, GLUL, IL6ST and repressed IL-8 expression in both continuous and pulsatile treatment. However, fold induction of FKBP5 and GILZ were lower with pulsatile Hc than when given continuously ($p<0.02$, $p<0.007$ respectively). No differences were observed in expression of GLUL, IL6ST and IL-8 between the two delivery modes.
HeLa cells (n=3) showed a significantly greater degree of apoptosis in the pulsatile treatment group compared to the continuous group ($p<0.001$), although in primary T cells, no difference was seen.
Bioinformatic analysis identified common binding sites for the transcription factor CCAAT Displacement Protein (CDP) in the differentially regulated target genes found in the microarray. The importance of CDP was explored in HeLas using a luciferase reporter gene under the control of MMTV promoter, known to be both GC responsive and contain CDP binding sites. A significantly higher MMTV-Luc activity was observed with continuous Hc compared to pulsatile ($p< 0.001$), confirming the in silico findings.
Conclusion- We present data, on two cell types, supporting the biological relevance of altered Hc dynamics to target cell responses. These findings help us better understand the importance of the natural ultradian cortisol pulsatility and has implications for therapeutic glucocorticoid drug design and also for the administration of glucocorticoids therapeutically.

WILLIAMS-BEUREN SYNDROME CHROMOSOME REGION 22 (WBSCR22) IS A NOVEL MODULATOR OF GLUCOCORTICOID SENSITIVITY.

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Background- Williams-Beuren syndrome (WBS) is caused by contiguous deletion of approximately 1.5Mb at Chr:7q11.23. It manifests as multi-system developmental disorder with various phenotypes including cardiovascular, cognitive and endocrine disorders such as hypothyroidism, hypercalcemia and osteoporosis. Importantly, diabetes mellitus and glucose intolerance have been reported in 75% of affected adult cases. The deleted region is thought to encode for 26-28 genes including WBSCR22. The function of *WBSCR22* is currently unknown but sequence homology suggests it encodes a putative methyltransferase protein.
Hypothesis- WBSCR22 is a novel glucocorticoid sensitivity gene which acts through the glucocorticoid receptor (GR) to alter GR functions.
Methods- To investigate whether WBSCR22 alters glucocorticoid sensitivity, GR transactivation and transrepression assays were performed in HeLa cells, using a luciferase reporter gene under the control of glucocorticoid (Gc)-responsive tyrosine amino transferase-3 (TAT3-Luc) and NF-kB responsive element (NRE-Luc) in the absence or presence of dexamethasone (Dex). GR specificity of WBSCR22 was also explored using TAT3-Luc assays in nuclear hormone receptor deficient HEK cells through exogenous expression of GR, mineralocorticoid receptor (MR), progesterone receptor (PR) and androgen receptor (AR). Data were analysed in triplicates and compared using independent sample t tests.
Results- GR activation by 10nM Dex significantly increased TAT3-Luciferase activity compared to the control ($p<0.001$) in HeLa cells. Overexpression of WBSCR22 significantly enhanced GR-mediated TAT-3 activation ($p<0.002$) in the presence of 10nM Dex. TNF-α (0.5ng/ml) maximally activated NF-kB luciferase reporter gene resulting in GR transrepression. This effect was impaired with higher TNF- α concentration (5ng/ml). Co-transfection of NRE-Luc and WBSCR22 transfection however, was not seen to modulate GR-mediated repression of NF-kB activity.
Dex, corticosterone, progesterone and dihydrotestosterone (DHT) elicited activation of TAT3 promoter in HEKs exogenously transfected with GR, MR, PR or AR. However, when WBSCR22 was added a robust induction of TAT3 promoter was observed which was unique to GR and not MR, AR or PR. This activation was found to be both ligand-independent ($p=0.001$) and ligand-dependant ($p<0.001$).
Conclusion - WBSCR22 specifically increases transactivation by GR of TAT3 promoter but does not affect GR-mediated transrepression. This will provide insight into the potential mechanism of action of WBSCR22 and characterise the function of *WBSCR22* gene to determine its potential importance in Gc related pathologies.

BDNF AND GLUCOCORTICOID RECEPTOR SIGNALING AND CROSSTALK

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Two cardinal signaling systems, the corticosteroid and the neurotrophins, are involved in scaling stress responses. Glucocorticoid (GC) is a hormone secreted by the adrenal gland under the control of the brain to orchestrate the physiological response to threats and stress. Brain-derived neurotrophic factor (BDNF) is secreted upon neuronal activity to strengthen the neuronal circuits. Both systems are interrelated because prolonged secretion of GC by administration or chronic stress reduces BDNF synthesis and function in the brain. In contrast, short-lasting and moderate elevation of GC levels rapidly induces BDNF expression and signaling in the brain. To explain these effects, we hypothesize that BDNF and GC undergo crosstalk between their respective receptors. Using microarray assays of primary cortical neurons, we analyzed the genome-wide effects of treatments with dexamethasone (Dex), a synthetic GC in comparison with BDNF. Whereas Dex or BDNF treatments alone regulated independent sets of genes, co-treatment with Dex and BDNF targeted and enhanced a separate set of genes. Among the regulated genes, the expression of the neuropeptide CRH (corticotrophin releasing hormone) was down-regulated by Dex, up-regulated by BDNF, but hyper-repressed by the combination of ligands. The BDNF receptor (TrkB) antagonist, K252a, abolished the transcriptional effects of BDNF upon Dex signaling though its nuclear receptor. CRH is the major brain-derived trigger of GC release. We found CRH expression is down-regulated and basal GC plasma level is reduced in BDNF and TrkB knockout mice. Administration of Dex (0.1 mg/kg) strongly repressed GC plasma levels in wild-type mice, but only weakly in TrkB and BDNF knockout mice. These results indicate crosstalk exists between GC and BDNF signaling. Using mass spectrometry of neurons treated with BDNF, we found that the GC receptor (GR) was rapidly phosphorylated at specific sites within the N-terminal domain by BDNF. Mutation of these phosphorylation sites changed the transcriptional profile of the GR in cortical neurons. Therefore, BDNF signaling can modify and amplify GR transcriptional activity in the brain, which provides a mechanism to account for the differential effects of GC levels upon stress and behavior.

THE ACTIN-BINDING PROTEIN, A ACTININ ALPHA 4 (ACTN4), IS A NUCLEAR RECEPTOR COACTIVATOR THAT IS REQUIRED FOR ESTROGEN RECEPTOR-MEDIATED TRANSCRIPTIONAL ACTIVATION IN MCF-7 BREAST CANCER CELLS

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Alpha actinins (ACTNs) are known for their ability to modulate cytoskeletal organization and cell motility by crosslinking actin filaments. Overexpression of ACTN4 has been linked to breast cancer, but the underlying mechanisms remain largely unknown. We show here that ACTN4 harbors a functional LXXLL receptor interaction motif, interacts with nuclear receptors in vitro and in mammalian cells, and potently activates transcription mediated by nuclear receptors. While ACTN4 potentiates estrogen receptor alpha (ERα)-mediated transcription in transient transfection reporter assays, knockdown of ACTN4 decreases it. In contrast, histone deacetylase 7 (HDAC7) inhibits estrogen receptor alpha (ERα)-mediated transcription. Moreover, the ACTN4 mutant lacking the CaM (calmodulin)-like domain that is required for its interaction with HDAC7 fails to activate transcription by ERα. Chromatin immunopreciptation (ChIP) assays demonstrate the association of ACTN4 and HDAC7 with the ERα target promoter pS2 gene, indicating that ACTN4 and HDAC7 directly regulate the expression ERα target gene. Knockdown of ACTN4 by siRNA significantly decreases the expression of ERα target genes, pS2 and PR and cell proliferation of MCF-7 breast cancer cells with or without hormone, whereas knockdown of HDAC7 exhibits opposite effects. Interestingly, overexpression of wild-type ACTN4, but not the mutants defective in interacting with ERα or HDAC7, resulted in increases in pS2 and PR mRNA accumulation in a hormone-dependent manner. In summary, we have identified ACTN4 as a novel, atypical coactivator that regulates transcription networks to control cell growth.

TARGETING EGFR-ASSOCIATED SIGNALING PATHWAYS IN NON-SMALL CELL LUNG CANCER CELLS: IMPLICATION IN RADIATION RESPONSE

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Several studies have demonstrated solid evidence for the potential value of targeting epidermal growth factor receptor (EGFR) signaling to enhance the anti-tumor activity of radiation. However, therapeutic resistance has emerged as an important clinical issue. Here, we investigated whether strategies for targeting EGFR-associated downstream signaling would radiosensitize a panel of non-small cell lung cancer (NSCLC) cell lines. Inhibition of K-RAS using RNA interference attenuated downstream signaling and increased radiosensitivity of A549 and H460 cells, while inhibition of EGFR did not. A549 cells harboring a K-RAS mutation at codon V12 were radiosensitized by siRNA targeting this codon. H460 cells having mutation at codon V61 was radiosensitized by siRNA targeting of this mutation. K-RAS siRNA did not radiosensitize H1299 cells possessing wild-type K-RAS.
Inhibition of the PI3K-AKT-mTOR pathway led to significant radiosensitization of the two cell lines, while selective inhibition of ERK signaling did not. Inhibitors targeting the PI3K-AKT-mTOR pathway also abrogated G_2 arrest following irradiation and induced γH2AX foci formation. A dual inhibitor of Class I PI3K and mTOR effectively increased radiosensitivity of A549 and H460 cells. Inhibition of PI3K-AKT signaling was associated with downregulation of DNA-PKs. While apoptosis was the primary mode of cell death when cells were pretreated with LY294002 or AKT inhibitor VIII, cells pretreated with rapamycin or PI-103 showed mixed modes of cell death, including apoptosis and autophagy.

Our results suggest possible mechanisms for counteracting EGFR prosurvival signaling implicated in radioresistance and offer an alternative strategy for overcoming resistance to EGFR inhibitors used in combination with irradiation. (Work supported by BAERI #2007-2001193 &NRF#1E00061from Korean Ministry of Education, Science & Technology)

MODELING OF THE ARYL HYDROCARBON RECEPTOR LIGAND BINDING POCKET AND IDENTIFICATION OF NEW LIGANDS BY VIRTUAL LIGAND SCREENING

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The Aryl Hydrocarbon Receptor (AhR) is a ligand-activated transcription factor and a member of the bHLH/PAS (basic Helix-Loop-Helix/Per-Arnt-Sim) family of proteins. The AhR is activated by a variety of synthetic and natural compounds, including halogenated aromatic hydrocarbons such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). The activated AhR has been implicated in immune suppression, disruption of endocrine signaling and alteration of cell growth. Significant differences have been observed among various species in the ability of ligands to bind the AhR and induce biological responses. While the PAS domain of the AhR is known to be responsible for ligand binding, the structural basis for the inter-species differences remains poorly understood. To address this, we constructed homology models of the AhR PAS domain from several different species using available structural information from other closely related proteins. Using these models, many of the intra- and inter-species differences in TCDD binding were structurally characterized using Molecular Docking. Virtual ligand screening of chemical libraries using the mouse AhR ligand binding domain model resulted in the identification of novel putative ligands. We will present data involving characterization of the newly identified ligands and their AhR-dependent biological effects.

IN SILICO ANALYSIS IN COMBINATION WITH HIGH-THROUGHPUT INTERACTOMICS IDENTIFIES PUTATIVE NOVEL BROWN/WHITE SPECIFIC PPARγ COREGULATORS.

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Adipose tissue development is a process which involves the concerted action of several transcription factors together with their coactivators and corepressors. The peroxisome proliferator activated receptor γ (PPARγ) is considered to be one of the master regulators of adipocyte differentiation. The presence of two functionally distinct types of adipose tissue, white and brown (WAT and BAT), requires an even more complex regulation of adipose tissue development. Here we present an in silico analysis based on existing mRNA expression profiles of brown and white adipose tissue samples, combined with the list of nuclear receptor coregulators given by the NURSA site. Strikingly, 68 coregulators displayed higher expression in WAT compared to BAT, while only 9 genes displayed a higher expression in BAT than in WAT. Furthermore, we performed a peptide microarray analysis to study the interaction between PPARγ and 155 peptides derived from LxxLL/LxxxIxxxL containing coregulators. Of the ligand dependent interacting peptides, the peptides derived from RIP140, NCOR1 and SMRT displayed differential expression between BAT and WAT. It may therefore be hypothesized that both NCOR1 and SMRT might be involved in determining the distinctive properties of WAT and BAT, for instance by repressing brown-specific PPARγ target genes in WAT, as already has been shown for the repression of UCP1 by RIP140 in WAT.
To conclude, the in silico analysis resulted in a list of 77 NR coregulators, of which a large part has not been linked previously to PPARγ. Further interaction studies may reveal PPARγ coregulators which are involved in determining brown/white adipocyte identity.

HAIRLESS - A NOVEL TRANSCRIPTIONAL REGULATOR OF ADIPOCYTE DIFFERENTIATION

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Uncontrolled adipose accretion (obesity) caused by an increasingly predominant sedentary lifestyle represents a key risk factor for a plethora of life-threatening clinical complications including type 2 diabetes mellitus and thus accounts for seemingly measureless world health costs. Expansion of the adipose depots occurs through growth, enhanced proliferation and differentiation of fat cells (adipocytes) or their progenitors, respectively. In fact, much attention has been devoted to identification of factors that inhibit or promote adipocyte differentiation, modulation of which might be an interesting avenue to combat obesity. The aim of this study is to identify novel players in adipogenesis, in particular factors that regulate epigenetic events ensuring the dramatic changes in the transcriptional profile. We initially focused on a novel class of proteins containing JmjC-domains that were attributed histone demethylating activity and that have been recently implicated in cellular differentiation. To assess the involvement of possible histone demethylating enzymes, mRNA expression levels of the 27 JmjC-domain containing genes were assessed over the course of adipogenic differentiation of 3T3-L1 cells. One transcript in particular, hairless, showed a very rapid upregulation early after initiation of differentiation and a sharp decrease after peaking, declining back to almost basal levels. Hairless is a known corepressor and interacts with nuclear receptors such as the thyroid hormone receptor, the vitamin D receptor and the retinoic acid receptor-related orphan receptors. Based on the phenotype of hairless mutant mice and humans, hairless has an important role in the skin. Our group has found that knockdown experiments in 3T3-L1 cells showed an absolute requirement of hairless for adipogenesis to occur. Important transcriptional events that ensure adipocyte differentiation to proceed are not taking place. Furthermore, forced expression of hairless in non-commited mesodermal cell lines potentiates their capacity to become adipocytes. Most importantly, hairless knockout mice showed a severe metabolic phenotype and markedly reduced adipose tissue formation. A cell autonomous differentiation defect was identified in preadipocytes and fibroblasts isolated from these mice. Detailed future investigations of the molecular mechanism will elucidate how hairless is exerting its proadipogenic effects. Our data provide a novel potential mechanism required for adipogenesis, thus possibly offering new therapeutic avenues that may interfere with unfavourable energy homeostasis in diabetic patients.

MECHANISMS OF GLUCOCORTICOID-INDUCED MUSCLE ATROPHY AND INSULIN RESISTANCE

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Glucocorticoids are steroid hormones involved in diverse cell type-specific physiological processes, including glucose, lipid, and protein metabolism, and are powerful anti-inflammatory agent. Extensive exposure to glucocorticoids can cause muscle atrophy and insulin resistance. Although these side effects are well documented, mechanisms governing glucocorticoid-induced muscle atrophy and insulin resistance are largely unknown. Our goal is to identify glucocorticoid-regulated primary genes that are responsible for inducing muscle atrophy and insulin resistance. Using chromatin immunoprecipitation-high throughput DNA sequencing (ChIPseq), we have identified 2252 glucocorticoid receptor (GR) binding regions, corresponding to 1608 genes, in mouse C2C12 myotubes. Microarray confirms that 142 of these 1608 genes are regulated by glucocorticoids. Among these potential primary GR targets, *FoxO3A* and *PIK3R1* are two genes with known connection to muscle atrophy and insulin signaling pathway, respectively. Reported by others, constitutively active *FoxO3A* alone can cause muscle atrophy. Here, we show that glucocorticoids induce *FoxO3A* transcript and active protein levels. The expression of luciferase reporter that harbors GR binding regions of *FoxO3A* is significantly induced by glucocorticoids. Intriguingly, mutating a single nucleotide in one of the GR binding regions, glucocorticoid-dependent reporter gene expression is abolished entirely, confirming the validity of *FoxO3A* GRE. Moreover, glucocorticoid treatment increases the acetylation of histone H3 and H4 in the surroundings of *FoxO3A* GR binding regions. Furthermore, overexpressing *PIK3R1*, the regulatory subunit of *PI3K*, has been reported to decrease insulin sensitivity; however, its role in muscle atrophy has not been tested. Here, we show that *PIK3R1* overexpression in C2C12 myotubes leads to a 35% decrease in cell diameter, which strongly suggests its role in glucocorticoid-induced muscle atrophy. Overall, we have identified several genes, activated by GR concertedly, modulate protein metabolism. These results shall benefit future therapeutic development for better pharmaceutical interventions.

MODULATION OF GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL ACTIVITY VIA BDNF-DEPENDENT PHOSPHORYLATION

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Glucocorticoid receptor (GR) is a ligand-dependent transcription factor that is activated by glucocorticoid hormones (GC). Despite its ubiquitous expression, GR shows a remarkable ability to regulate gene expression in a cell type specific manner. Such tissue specificity of GR action is likely mediated through signal-dependent post-translational modifications of the receptor, such as phosphorylation. The brain is a major GC site of action, yet the impact of GR phosphorylation in facilitating GC action in neurons remains largely uncharacterized. We now report that primary neurons stimulated with the Brain Derived Neurotrophic Factor (BDNF), the ligand for the TrkB tyrosine kinase receptor, induce phosphorylation of GR. Using (MALDI-TOF) mass spectrometry, we identified two previously uncharacterized sites, Serine 155 (S155) and Serine 287 (S287) within the N-terminal transcriptional regulatory domain of rat GR that are phosphorylated upon BDNF treatment. Here, we address how BDNF signaling modulates GR function.

To investigate BDNF and GR crosstalk, we used a microarray approach to determine the genes that are regulated by GC and BDNF in primary rat cortical neurons. BDNF and Dexamethasone (Dex) co-treatment regulated more than twice the amount of genes compared to any single treatment. Interestingly, 45% and 55% of those genes in the BDNF + Dex treated group were uniquely induced and repressed, respectively, only upon co-treatment, suggesting that a distinct genetic program is elicited by co-treatment. Furthermore, the magnitude of a subset of the Dex-induced (and repressed) genes was amplified upon treatment with BDNF, suggesting a BDNF-dependent modification of select GR responsive genes. The effect of BDNF signaling on the GC-dependent transcription was validated by RT-qPCR on a select group of genes. Moreover, the effect of BDNF on GR activity appears dependent upon GR phosphorylation at S155 and S287, since a serine to alanine double mutation (GR S155A/S287A) abolished much of the gene expression induced by wild type GR at endogenous target genes in primary neurons.

Using GR phospho-specific antibodies, we are currently investigating the molecular mechanisms sensitive to BDNF signaling that are employed by GR to modify transcription. Although GC and BDNF are often viewed as antagonistic pathways in chronic stress and pathological conditions in the brain, we propose that BDNF signaling acts as an endogenous amplifier of GC response in neuronal cells through a GR phosphorylation-dependent mechanism.

LIVER X RECEPTOR (LXR) REGULATES HUMAN ADIPOCYTE LIPOLYSIS

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The Liver X Receptor (LXR) is an important regulator of carbohydrate and lipid metabolism in humans and mice. We have recently shown that activation of LXR regulates cellular fuel utilization in adipocytes. In contrast, the role of LXR in human adipocyte lipolysis – the major function of human white adipocytes – has not been clear. In the present study, we stimulated in vitro differentiated human adipocytes with the LXR agonist GW3965 and observed an increase in basal and hormone-stimulated lipolysis. Microarray analysis of human adipocyte mRNA following LXR activation resulted in altered gene expression of several lipolysis-regulating proteins which was also confirmed by quantitative real-time PCR. In addition, we investigated the protein expression, phosphorylation and intracellular localization. Using chromatin immunoprecipitation we show that upon activation LXR is recruited to several promoters of lipolysis-regulating genes. We propose that LXR is upregulating human adipocyte lipolysis via two distinct mechanisms. First by down-regulating the expression of the lipid droplet-coating proteins Perilipin and Cell death-inducing DNA fragmentation factor, α subunit-like effector C (CIDEC) thereby increasing the access of lipases to stored triglycerides and enhancing basal lipolysis. Secondly, LXR activation downregulates phosphodiesterase 3B and upregulates adenylate cyclase 3 thereby increasing the intracellular levels of cAMP. By using siRNA treatment we also show that LXRα and LXRβ have different roles in regulation of human adipocyte lipolysis. In conclusion, we demonstrate a new role for LXR in human white adipocytes and provide a putative mode of action.
This study was supported by the Swedish Research Council, NovoNordisk, Swedish Diabetes Association, EndoMet, Karolinska Institutet, the European Union [HEPADIP (LSHM-CT-2005-018734), ADAPT (HEALTH-F2-2008-201100], COST action BM 0602, Magn Bergvalls and Åke Wibergs foundations.

HIGH THROUGHPUT PHAGE DISPLAY IDENTIFIED NOVEL ERβ INTERACTORS

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Numerous studies on estrogen signaling have undoubtedly led to the improvement in treatment for breast cancer and other endocrine related disorders. Since 1996, the discovery of the second estrogen receptor, ERβ, has brought about another dimension of complexity upon this picture of estrogen signaling. Its role in cell physiology remains a controversial topic. The challenges are mainly due to the lack of a reliable antibody for this particular protein and the differences in cellular contexts used to analyze ERβ functions. In the present study, we have successfully applied a phage display followed by an exon microarray to identify novel ERβ interacting proteins in a cell-free manner. Briefly, estradiol-bound ERβ was incubated on an ERE-coated plate; a phage library which expressed the breast cDNA was added; the peptides expressed on the phage surface allowed binding to the immobilized receptor; the cDNA from bound phage were extracted and subjected to exon microarray analysis. With p-values less than 0.05, we have identified 845 proteins at low-stringent threshold (FC > 1.01) and 372 proteins at high-stringent threshold (FC > 2.00). 5 out of 7 novel ERβ interactors from our screen were confirmed using mammalian two hybrid assays. Currently, we are investigating a subset of the novel interactors which are involved in transcriptional regulation. Moving forward in this study, we wish to deliver a better understanding on the molecular functions of ERβ as part of the complex transcriptional machinery. Ultimately, this will give birth to new potential therapeutic targets for endocrine related diseases.

THE ROLE OF THE RETINOID X RECEPTORS IN STEM CELL DIFFERENTIATION

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Vitamin A is essential for proper embryonic development. Both natural and synthetic retinoids are powerful regulators of cell growth and differentiation, and treatment of tissue cultures with these compounds enhances the differentiation of pluriopotent stem cells into specific cell lineages. Retinoic Acid (RA) induces cellular differentiation by activating the retinoid receptors which regulate gene transcription through binding to enhancers or promoters of the genes that they govern. RA can bind to both the Retinoic Acid Receptor (RAR) and the Retinoid X Receptor (RXR) and is believed to exert its effects through RAR/RXR heterodimers. It is currently unknown how much the RXR contributes to RA induced cellular differentiation. Preliminary results in our lab using pluripotent stem cells and an RAR selective ligand demonstrate that activation of RARs alone does not achieve the level of cellular differentiation that RA does. However, using a selective ligand to activate RXR alone achieves greater efficacy than RA. Our preliminary data using embryonic stem cells is promising and has confirmed that RXRs are the key regulators in stem cell differentiation. Our study will allow us to uncover novel pathways of RA signalling and to elucidate whether RXR is acting alone as a homodimer, or in conjunction with RAR as a heterodimer, as well as the target genes of the RXRs involved in cellular differentiation. By increasing our understanding of transcriptional regulation in the early stages of stem cell differentiation, we can learn how to direct lineage specification and to generate sufficient amount of tissues to use in the treatment of debilitating diseases.

PPARγ2 CONTROLS BONE MASS, MARROW MESENCHYMAL STEM CELL DIFFERENTIATION AND ENERGY METABOLISM IN BONE

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Bone loss with aging results from attenuated and unbalanced bone turnover and is associated with a decreased number of bone-forming osteoblasts (OB), increased number of bone-resorbing osteoclasts (OC), and increased number of adipocytes (AD), in the bone marrow. These changes are associated with changes in marrow MSC lineage allocation and correlate with increased expression of PPARγ2, decreased expression of pro-OB Runx2 transcription factor, and increased expression of RANKL, a cytokine supporting osteoclastogenesis. Similarly to aging, PPARγ agonists TZDs cause bone loss, increase fat content in bone, and increase fracture risk in humans, specifically in postmenopausal women and elderly. In animals, TZDs induce bone loss by suppressing new bone formation and increasing bone resorption. Aging and estrogen deficiency sensitize bone to the TZD-induced loss by increasing bone resorption.
PPARγ2 is a key regulator of bone cell development and activity. It directs MSC differentiation toward the AD and at the expense of OB lineage. Circadian protein Nocturnin, an accessory PPARγ protein, regulates MSC lineage allocation. PPARγ2 pro-AD activity is transcriptional in nature and involves binding to PPAR response elements (PPRE), whereas anti-OB activity is PPRE-independent and involves suppression of Wnt, TGFβ/BMP and IGF-1 signaling followed by suppression of OB-specific transcriptional regulators. Pro-AD and anti-OB activities of PPARγ can be separated with selective ligands, reinforcing a notion of distinct regulatory pathways controlling these activities.
PPARγ2 regulates the expression of a number of genes associated with the stem cell phenotype and formation of a micro-environment supporting hematopoiesis. It negatively regulates the expression of “stemness” genes, which include ABCG2, Egfr, CD44, Kitl, SDF-1, LIF and LIFR, and hematopoiesis-supporting genes. Thus, as a result of increased PPARγ expression with aging or activation with TZDs, MSCs lose their ”stemness” and are more prone to AD and less prone to OB differentiation.
Bone fat (YAT) occupies a significant portion of bone marrow cavity. Its metabolic activity is unknown. YAT depot increases in its volume in response to estrogen deficiency and in metabolic diseases, such as obesity, diabetes, and anorexia nervosa. Gene expression profiling shows that YAT may display great plasticity in acquiring WAT-like or BAT-like phenotype in response to systemic changes in energy metabolism. This may be of local importance to bone, as well as to the systemic control of energy metabolism, e.g. in conditions of functional impairment of other fat depots.

ANTIINFLAMMATORY EFFECTS OF A NOVEL AGONIST LIGAND FOR THE NUCLEAR RECEPTOR LRH-1 IN INFLAMMATORY BOWEL DISEASE.

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Nuclear hormone receptors regulate diverse metabolic pathways. The orphan nuclear receptor LRH-1 is essential for bile acid biosynthesis in the liver and can induce glucocorticoid production in the intestine in response to inflammatory stress. Our previous results have shown that an unusual phosphatidylcholine species with two saturated 12 carbon fatty acid acyl side chains (dilauroyl phosphatidylcholine, DLPC) is an LRH-1 agonist ligand. Here we show that DLPC has remarkable antiinflammatory effects in two mouse models of chemically induced inflammatory bowel disease (IBD). In 2, 4, 6-trinitrobenzene sulfonic acid (TNBS)-induced colitis, preventative DLPC treatment induces local glucocorticoid biosynthetic enzymes and antiinflammtory cytokines, and decreases proinflammatory cytokines in the colon. Moreover, in dextran sodium sulfate (DSS)-induced colitis, therapeutic DLPC treatment also induces local glucocorticoid biosynthetic enzymes and antiinflammatory cytokines, and decreases proinflammatory cytokines in the colon. These effects are lost in intestine specific *Lrh-1* knockouts. Interestingly, DLPC treatment in DSS-induced colitis also induces Twist1 and 2, negative regulators of NF-kB complex in the colon. These findings identify an LRH-1 dependent phosphatidylcholine signaling pathway that regulates intestinal immunity in response to immunological challenges by triggering local corticosteroid production and negative regulators of NF-kB signaling pathway.

THE COACTIVATOR SRC-1 IS AN ESSENTIAL COORDINATOR OF HEPATIC GLUCOSE PRODUCTION

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Gluconeogenesis makes a major contribution to hepatic glucose production, a process critical for survival in mammals. In this study, we identify the p160 family member, SRC-1 as a key coordinator of the hepatic gluconeogenic program in vivo. SRC-1 null mice displayed hypoglycemia secondary to a deficit in hepatic glucose production, as assessed by hyperinsulinemic-euglycemic clamp. Selective re-expression of SRC-1 in the liver restored blood glucose levels to a normal range. SRC-1 was found induced upon fasting to coordinate in a cell-autonomous manner, the gene expression of rate-limiting enzymes of the gluconeogenic pathway including pyruvate carboxylase (PC), fructose 1-6 bisphosphatase (FBP1) and phosphoenolpyruvate carboxykinase (PEPCK). At the molecular level, the main role of SRC-1 was to modulate the expression and the activity of C/EBPalpha through a feed-forward loop in which SRC-1 used C/EBPalpha to transactivate PC, a crucial gene for initiation of the gluconeogenic program. We propose that SRC-1, acts as a novel and critical mediator of glucose homeostasis in the liver by adjusting the transcriptional activity of key genes involved in the hepatic glucose production machinery.

"NON-INVASIVE IMAGING OF PANCREATIC INFLAMMATION IN MODELS AND PATIENTS"

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One roadblock to research on and therapy of type-1 diabetes is the lack of a non-invasive method to image the imitation and unfolding of disease. We have developed a method, based on magnetic resonance imaging of magneto fluorescent particles (MRI-MNP) that permits in murine T1D models: detection of insulitis, an assessment of insulitis severity, prediction of conversion to overt diabetes, and early assessment of a drug's efficacy in clearing islet inflammation. This technique has been exploited to identify a critical new pathway in T1D pathogenesis. Results from a just-completed small clinical trial argue for successful application of this technique to human T1D patients.

CRYO-ELECTRON MICROSCOPY STRUCTURE OF THE 100KDA FULL NUCLEAR RECEPTOR RXR/VDR HETERODIMER COMPLEX WITH ITS TARGET DNA.

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Until recently, structural studies of nuclear receptors have focused on their two individual core domains, the ligand-binding domain (LBD) and the DNA-binding domain (DBD), with the exception of the full retinoid X receptor (RXR) complex with the peroxisome proliferator-activated receptor (PPAR; Chandra et al., 2008). The presentation will address the architecture of the full nuclear receptor heterodimeric complex of the ligand-bound vitamin D receptor (VDR) and RXR bound to their DNA response element that comprises a direct repeat (DR3). The solution structure of the RXR/VDR/DNA complex has been determined by high-resolution single particle cryo electron microscopy (cryo-EM) whilst overcoming technical challenges related to the small molecular weight of the object (100kDa, to our knowledge the smallest complex studied by cryo-EM up to now). The structure reveals an open conformation rather different from the crystallized PPAR/RXR complex. The RXR and VDR LBDs and DBDs adopt a parallel organization, with the LBDs perpendicular to the DNA and positioned at the DNA 5'-end of the response element (Orlov et al., submitted). The DBD and LBD parts are connected through well-resolved hinges, without additional contacts between DBDs and LBDs, suggesting that the hinges provide independent links to the DBDs and the DNA and thereby stabilize the complex in a precise conformation on its target DNA. The present structure has important implications in the general architecture of nuclear receptors and in addition provides first molecular insights into the orientation of transcriptional co-regulators that are recruited to the area of transactivation helix H12. We will also present data on an entirely different complex bound to an inverted DNA repeat.

INFLAMMATORY LINKS BETWEEN OBESITY, DIABETES AND ENERGY EXPENDITURE

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Obesity is associated with chronic low-grade inflammation that negatively impacts insulin sensitivity. This involves infiltration of M1 polarized proinflammatory macrophages into fat tissue, as well as inflammatory events in liver. High fat diet can increase NFκB activation in mice, which leads to a sustained elevation in level of IκB kinase ε (IKKε) in liver, adipocytes and adipose tissue macrophages. IKKε knockout mice are protected from high fat diet-induced obesity, chronic inflammation in liver and fat, hepatic steatosis and whole-body insulin resistance. These mice show increased energy expenditure and thermogenesis on high fat diet compared to wild type mice. They maintain insulin sensitivity in liver and fat, without activation of the proinflammatory JNK pathway associated with obesity. Gene expression analyses indicate that targeted deletion of IKKε increases expression of the uncoupling protein UCP-1, reduces expression of inflammatory cytokines, and changes expression of certain regulatory proteins and enzymes involved in glucose and lipid metabolism. Thus, IKKε may represent an attractive new therapeutic target for obesity, insulin resistance, diabetes and other complications associated with these disorders.

IDENTIFICATION OF NOVEL TARGETS FOR PPARγ using TRANSCRIPTIONAL PROMOTER ONTOLOGY

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We have developed a novel high-throughput method for functional analysis of transcription by the Nuclear Receptor family. By combining the power of a validated cDNA expression library, covering the entire Nuclear Receptor Family, with pathway specific promoter-reporter libraries, we can specifically evaluate the transcriptional regulation of genetic pathways by any NR in a given context (i.e., in the presence or absence of ligand, in different cell lines etc.).
A subgroup of the NRs, the Peroxisome proliferator-activated receptors (PPARs) are activated by fatty acids and are integrally involved in lipid and glucose metabolism. PPARγ is a master regulator of adipogenesis and a central player in the pathophysiology of Type 2 diabetes and obesity. PPARγ is also the molecular target for the widely prescribed thiazolidinedione (TZD)-class of insulin sensitizers. Despite their efficacy in glycemic control, TZDs are associated with various adverse side effects, including weight gain, edema, and liver and cardiovascular toxicity, which limits their clinical use. The molecular mechanisms by which TZDs mediate their insulin sensitizing effects are still poorly understood, but it is clear that adipose tissue plays a central role in their action.
Using our functional screen for NR-mediated promoter regulation, we have identified novel targets of PPARγ which might provide insight into the mechanism by which TZDs and PPARγ mediate their insulin sensitizing effects.

GPR120 IS AN OMEGA-3 FATTY ACID RECEPTOR MEDIATING POTENT ANTI-INFLAMMATORY AND INSULIN SENSITIZING EFFECTS.

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We find that the G protein-coupled receptor 120 (GPR120) functions as a receptor/sensor for the omega-3 fatty acids, DHA and EPA. Stimulation of GPR120 with omega-3 FAs or a chemical agonist, causes inhibition of TNF-α and lipopolysaccharides (LPS)-induced inflammatory signaling responses in monocytic RAW 264.7 cells and in primary intraperitoneal macrophages. These effects proceed through a β-arrestin2/TAB1 dependent mechanism, which leads to inhibition of TAK1. All of these effects are abrogated by GPR120 knockdown. Since DHA-mediated GPR120 agonism induces anti-inflammatory effects *in vitro*, we fed high fat diets with or without omega-3 FA supplementation to wild type (WT) and GPR120 knockout mice. The omega-3 FA treatment inhibited inflammation and enhanced insulin sensitivity in WT mice, but was without effect in GPR120 knockout mice. In conclusion, GPR120 is a functional omega-3 FA receptor/sensor and mediates potent anti-inflammatory and insulin sensitizing effects.

STRUCTURAL MECHANISMS OF SIGNAL INTEGRATION IN RXR HETERODIMERS

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Allostery in RXR heterodimers is poorly understood, including signal integration from ligands for both receptors, the silencing of RXR by VDR or TR, and ligand signaling via activation of the unliganded partner receptor (e.g. the phantom ligand effect). Here, we show that an electrostatic tether links the paired helices 11 of heterodimers and provides a conduit for transmission of allosteric information. A second region of the dimer interface mediates silencing of RXR by TR.

Mutations of the electrostatic tethering amino acid RXRα E434 to other polar or charged amino acids effected signal integration with FXR, LXR, TR, or VDR, including differential loss or gain of function depending on the dimer partner and ligands. Treatment of RXR E434N/LXR with 9cisRA induced a substantial gain of function (150 fold activation) compared to wild-type heterodimer (10 fold activation). This effect was via the LXR coactivator-binding site, as shown by a second site suppressor mutant in the LXR AF2 surface, which fully blocked coactivator binding to LXR, and activation by 9cisRA. This suggests a model whereby ligand stabilization of helix 11 transmits allosteric information to the partner receptor helix 11, modulating helix 12 dynamics in the partner receptor.

However, RXR 434 mutants were not able to restore responsiveness with TR or VDR, suggesting a different mechanism for silencing. We crystallized the RXRα/TRβ LBD heterodimer,showing RXR in an inactive conformation, despite the presence of coactivator peptide and RXR ligand. Buried in the center of the helix 11 dimer interface, RXR P423 is shifted by TR, causing a twist in the center of RXR helix 11. This twist is transmitted to helix 6, which lies across the top of the ligand binding pocket in the hydrophobic core of RXR. Finally, the twist in helix 6 W305 induces a clash with helix 3 L376 inducing dramatic shift of helix 3 that opens the ligand binding pocket, preventing both ligand and coactivator binding in this inactive conformation. These results were confirmed with H/D exchange mass spectrometry, showing reduced exchange in RXR helices 3 and 11 in the presence of excess RXR ligand and coactivator peptide. This suggests that the active conformation of RXR in the presence of TR may be conformationally constrained. In conclusion, our work defines two distinct regions of the dimer interface that regulate integration of signals from both dimer partners versus the silencing of RXR by TR.

METABOLIC FLEXIBILITY, STRESS AND INFLAMMATION

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An increase in subclinical inflammation is frequently associated with obesity. Acute phase reactant proteins, such as C-reactive protein (CRP), α1 acid-glycoprotein (α1 AG) and serum amyloid A (SAA) are clinically chronically elevated and are contributing factors towards metabolic dysfunction associated with the metabolic syndrome and are generally potently repressed by activation of PPARγ. We analyzed the plasma levels of three proteins that we have previously been identified as major secretory proteins from adipocytes We picked representatives of three groups of immune-related proteins: the classical acute phase reactant SAA3, α1 AG which is an acute phase reactant in the liver but which may have different functions in adipose tissue, and adipsin / complement factor D, a member of the alternative complement fixation cascade. Proinflammatory conditions, such as lipopolysaccharide and high fat diet lead to an increase in SAA3, have limited impact on α1 AG and decrease plasma adipsin levels. Similar trends are observed in the metabolically challenged ob/ob background, whereas mouse models that are protected against diet-induced challenges, such as adiponectin overexpressing mice, as well as PPARγ agonists display lower SAA3 levels and higher adipsin levels compared to controls. Fasting conditions are associated with a dramatic increase in SAA3 levels. These results further stablish the potent anti-inflammatory potential of PPARγ.

MECHANISTIC AND PHYSIOLOGICAL ROLES OF COREGULATORS IN TRANSCRIPTIONAL REGULATION BY STEROID RECEPTORS

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After hormone-activated steroid receptors bind to regulatory sites in their target genes, they recruit a large number of coregulators to modulate chromatin structure and regulate the recruitment and activation or repression of RNA polymerase II and its associated transcription factors. The intricate choreography of occupancy by these many coregulators on the target gene promoter has been thoroughly documented for a few target genes and suggests a complex and carefully coordinated series of functionally important interactions among many coregulators on the promoter. Furthermore, we and others have observed that individual coregulators are required for hormonal regulation of some but not all target genes that are regulated by a specific steroid hormone. In the context of these findings, the Stallcup lab is addressing several questions about the mechanistic and physiological roles of various coregulators in the hormone-driven regulation of target gene expression: 1) We are using RNA interference coupled with chromatin immunoprecipitation on selected target gene promoters to dissect the functional relationships by which one coregulator controls promoter occupancy by other coregulators; this process should elucidate the mechanisms that lead to establishment of an active transcription complex or a repressed state on the promoter in response to hormone. 2) We are exploring the mechanisms responsible for promoter-specific coregulator requirements. 3) We are defining subsets of hormone regulated target genes that require a specific coregulator and exploring the potential physiological implications of coregulator control of specific subsets of steroid hormone regulated target genes.

IN VITRO IDENTIFICATION OF SELECTIVE ANDROGEN RECEPTOR MODULATORS USING AN AR CONFORMATION CHANGE ASSAY

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The androgen receptor (AR) is expressed in many human tissues, controlling diverse physiological processes, and is often involved in disease. There is a significant clinical need for drugs to treat AR-related diseases, but it has proven difficult to treat disease in one tissue without side-effects in others. For instance, systemic inhibition of AR prevents the growth of prostate cancer, but it also results in bone and muscle loss. We created a cell-based assay to monitor AR conformation change and successfully used the assay in screens to identify anti-androgens with novel, non-competitive mechanisms of action. Here, we have adapted this assay to identify cell-type specific AR agonists and antagonists, in an effort to create an in vitro screen for tissue selective AR modulators (SARMs). We expressed the AR conformation change reporter in cell lines derived from clinically relevant AR-expressing tissues, and screened a chemical library for compounds that matched desired clinical profiles. Importantly, we identified compounds that inhibit AR activity selectively in prostate cells, which may reduce the side-effects associate with systemic anti-androgen therapy. Concurrently, we are developing a rat model to assess the tissue selective activity of AR in response to potential SARMs. The model uses the transcription of AR-regulated genes in multiple male and female tissues to develop a profile of AR activity in each tissue. We hope to validate this model using known SARMs and, in the near future, use it to test potential SARMs identified using the AR conformation change assay.

GENOME-WIDE CHROMATIN REMODELING DURING ADIPOGENESIS

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Adipogenesis is a tightly controlled process that involves an intricate network of transcription factors acting at different time points during differentiation. The peroxisome proliferator-activated receptor γ (PPARγ) is key player in this process; however, numerous other transcription factors, most notably members of the CCAAT/enhancer binding protein (C/EBP) family, have been shown to be critically involved. We have used DNase-seq to investigate genome-wide chromatin remodeling during adipogenesis. The temporal profile of DNase hypersensitive sites (DHS) representing open chromatin regions, can be clustered into four distinct clusters. Interestingly, during the first few hours following addition of adipogenic inducers there are dramatic changes in the occurrence of DHS. Some of these sites are unique to the early stages of adipocyte differentiation, whereas others persist in the mature adipocytes. Interestingly, we identify ~1000 transcription factor hotspots where multiple transcription factors bind at overlapping sites few hours after stimulation with the adipogenic cocktail and we show that C/EBPβ is required for the establishment of many of these hotspots. Our data furthermore indicate that different pioneering factors may act early in adipogenesis at PPARγ target sites to open the chromatin structure and assist recruitment of PPARγ.

A DISTINCT CLASS OF NUCLEAR RECEPTOR ALTERNATE SITE MODULATORS (NRAMS) THAT TARGET A NOVEL ANDROGEN RECEPTOR REGULATORY MECHANISM INVOLVING FKBP52 AND B-CATENIN

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Drugs that target novel surfaces on the androgen receptor and/or novel AR regulatory mechanisms are promising alternatives for the treatment of hormone refractory prostate cancer (HRPC). The 52 kDa FK506 Binding Protein (FKBP52) has been shown to be an important positive regulator of AR in cellular and whole animal models and represents an attractive target for the treatment of prostate cancer. We have identified a surface region on the androgen receptor (AR) hormone binding domain that, when mutated, displays a greater dependence on FKBP52 for normal function. Interestingly, the BF3 surface is also hypothesized to participate in the interaction of AR with the co-activator β-catenin, and co-expression of FKBP52 and β-catenin act in synergy to up-regulate both hormone-dependent and hormone-independent AR function. In addition, we have developed a series of small molecules that effectively inhibit the FKBP52 regulation of AR function and the synergistic up-regulation of AR function by FKBP52 and β-catenin. Surface plasmon resonance studies have confirmed that these inhibitors disrupt the regulation of AR by FKBP52 and β-catenin through interaction with the AR hormone binding domain. We have demonstrated that these novel compounds do not compete with hormone for binding the hormone binding pocket, nor do they compete with coactivator peptide for binding AF2. In addition, we have shown that these compounds inhibit AR function by preventing hormone-dependent dissociation of the Hsp90-FKBP52-AR complex which results in less hormone-bound receptor in the nucleus. Preliminary assays in early and late stage prostate cancer cells have demonstrated our novel compounds inhibit both prostate specific antigen expression and androgen-dependent proliferation. In summary, we have identified a putative FKBP52 interaction surface on the AR hormone binding domain and identified a series of small molecules that inhibit AR function by targeting that surface. This class of compounds, now termed NR alternate site modulators (NRAMs), would be useful in hormone-resistant prostate cancer, where coregulator overexpression and other mechanisms result in receptor activation in the absence of exogenous ligands. In addition, we have identified a novel functional interaction between FKBP52 and β-catenin, which has clear implications in prostate cancer progression to the hormone refractory state.

VARIATION IN GLUCOCORTICOID RECEPTOR BINDING SEQUENCE AND ITS EFFECT ON STRUCTURE

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DNA acts as a sequence-specific allosteric ligand that influences glucocorticoid receptor (GR) structure and function (Meijsing, Pufall et al. (2009) Science 324:407). Among the suggestions of this finding are that certain GR binding sequences (GBSs) might direct cell-type specific activity of GR. To explore this, we performed ChIP-seq of GR in the presence of dexamethasone in three different cell lines: A549 lung carcinoma, U2OS osteosarcoma, and NALM-6 preB acute lymphoblastic leukemia. As expected, the position weight matrix-derived 15bp GR binding motif of inverted hexameric half-sites separated by three base pairs conformed to the canonical motif, and differed little between cell lines. However, individual GBSs exhibited remarkable sequence diversity, and were found rarely to be used repeatedly. Further, the presence of a canonical GBS within a GR binding region was highly cell type-dependent, ranging from 70% in U2OS to only 20% in NALM-6 cells, suggesting that GR might bind cell-specifically to non-canonical sequences. To examine directly the range of GR binding preferences, we used a microfluidic system to assay binding to a comprehensive library of random 8bp DNA sequences, which comprises all possible canonical GR half-sites among other sequences. These experiments demonstrated that although GR favors binding to a canonical site, it also has appreciable affinity for other sequences. To better understand how the structure of GR accommodates and responds to such diverse, but often subtle, changes in GBS, we took advantage of the sensitivity of nuclear magnetic resonance. Interestingly, even variations of non-contacted base pairs, including the spacer, appeared to influence the structure of the protein, indicating that DNA topology plays an important role in directing receptor structure. We conclude that GBSs comprise a very large set of distinct allosteric ligands, both canonical and non-canonical, and that the effects of DNA on conformation are not limited to directly contacted bases, suggesting that highly degenerate sequences can both bind and thereby influence the regulatory activity of GR.

NR COACTIVATORS: PHYSIOLOGY AND DISEASE.

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Nuclear receptors (NRs) are major regulators of growth, inflammation, and metabolism. NR coactivators, such as the SRC-1/p160 family of molecules, amplify nuclear receptor induction of gene expression by coordinately implementing the downstream subreactions required for efficient NR-dependent transcription, such as chromatin remodeling, initiation, elongation, alternative RNA splicing, and eventually, ubiquitinylation and turnover of the receptor-coactivator transcription complex itself. Thus, coactivators represent transcriptional 'master genes' that can play mediating roles in the development of many inherited and acquired hormone-related human pathologies.

We will present evidence that SRC-family coactivators control multiple facets of growth and carbohydrate metabolism in mammals. SRC-3 controls primary breast tumor growth. SRC-1 is complementary to SRC-3 in oncogenesis; SRC-1 regulates, not primary tumor growth, but distant breast cancer metastasis. Finally, SRC-2 is a metabolic gate-keeper for glucose release from liver and its absence causes a Von Gierke's Disease phenotype in mice. Recent work in mice in our lab has substantiated that SRC-2 also regulates fat absorption from the gut and storage to result in positive energy accretion/balance.

The coactivator class of molecules is already providing important new insights to human diseases and promises to map future experimental blueprints for attacking 'polygenic diseases' and to generate novel ideas for proteomic therapeutic interventions. Studies are now underway in our laboratory to discover novel ligands that can directly bind to SRC-family coactivators and inhibit coactivator function by causing premature degradation of the molecules or inhibiting their functional interactions with NRs or other co-coactivators.

IDENTIFICATION AND MECHANISM OF DECANOIC ACID AS A NATURAL MODULATING LIGAND OF PPARγ.

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Peroxisome proliferator–activated receptor-γ (PPARγ) is the molecular target of the thiazolidinedione (TZDs) class of antidiabetic drugs that have many side effects. PPARγ is also activated by long chain unsaturated or oxidized/nitrated fatty acids, but its relationship with the medium chain fatty acids remains unclear despite that the medium chain triglyceride oils (MCT oils) have been used to control weight gain and glycemic index. We show that decanoic acid (DA), a major component MCT oils, is a direct ligand of PPARγ. DA binds and partially activates PPARγ without leading to adipogenesis. Crystal structure reveals that DA occupies a novel binding site. Treatments with DA and its triglyceride form improve glucose sensitivity and lipid profiles without weight gain in diabetic mice. Together, these results suggest that DA is a natural modulating ligand for PPARγ and the structure can aid in designing better and safer PPARγ-based drugs.

MULTIPLE NUCLEAR EXPORT SIGNALS IN THE THYROID HORMONE RECEPTOR: IMPLICATIONS FOR RESISTANCE TO THYROID HORMONE SYNDROME

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Thyroid hormone receptors α and β (TRα1 and TRβ1) function in the nucleus as transcription factors and direct the expression of specific genes in response to thyroid hormone. Both TRα1 and TRβ1 are shuttling proteins and their intracellular localization appears to be tightly regulated by a fine balance between nuclear import, nuclear retention, and nuclear export. We have carried out a detailed study on the signals involved in the nucleocytoplasmic shuttling of TRα1, by investigating the ability of separate domains of TR to target a heterologous protein either to the nucleus or cytoplasm. The four functional domains of TRα1 analyzed included the N-terminal transactivation domain which contains a novel nuclear localization sequence (NLS), the DNA-binding domain, the hinge domain which contains a classical bipartite NLS, and the ligand-binding domain (LBD). GFP-GST-GFP-tagged domain expression vectors were transiently transfected into HeLa cells. Fusion protein distribution patterns were visualized by live-cell fluorescence microscopy and scored for either a nuclear, cytoplasmic or whole cell distribution. Deletion studies indicated the presence of multiple nuclear export signals (NES's) in the LBD of TRα1. Minimal regions that encode a transferable NES were cloned N-terminal to the hinge domain NLS to verify the nuclear export function. Three NES's were identified in the LBD of TRα1, namely NESH3, NESH6 and NESH12. H3, H6 and H12 represent helices, 3, 6 and 12 respectively of TRα1. Classical NES's interact with the export factor CRM1. Our studies show that NESH3, H6, and H12 follow a CRM1- independent nuclear export pathway. NESH12 is fully conserved in TRβ1. The region containing NESH12 is also involved in a ligand-dependent transactivation function, termed AF2. This AF2 functional domain is highly conserved among the nuclear receptor family members, thus highlighting the importance of NES function for this region. A detailed mutagenesis study revealed that multiple hydrophobic amino acids participate in the nuclear export function of NESH12. NES function was additive, as a second copy of NESH12 increased the nuclear export of the fusion protein. Specific mutations introduced in one or more copies of NESH12 resulted in a significant decrease in nuclear export activity. The three NES's fall within the known hot spots of Resistance to Thyroid Hormone (RTH) mutations in TRβ1. One such mutation in NESH12, TRβ1 L454S showed moderately reduced nuclear export, compared to wild-type. This points to the possibility of reduced nuclear export as a contributing factor in RTH syndrome. The presence of multiple NLS's and multiple NES's and their conservation among the vertebrate species suggest that nucleocytoplasmic shuttling is crucial for the normal function of TR.

THE ROLE OF ANDROGEN RECEPTOR PHOSPHORYLATION IN CASTRATE RESISTANT PROSTATE CANCER.

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Prostate cancer is the second highest cause of male cancer related mortality. The main therapy for localised and metastatic disease is maximum androgen blockade.This approach has initial response rates of over 80% yet men relapse with castrate resistant prostate cancer (CRPC).Fluorescent in situ hybridisation was employed to asses AR amplification status and immunohistochemistry was employed to assess expression levels of AR, AR phosphorylated at serine residues 81, 94, 213,308, 650 and 791 in a cohort of 62 patients all with paired hormone naïve (HNPC) and CRPC tissue available for analysis.
Amplification of the AR was observed in 1.5% HNPC, this increased significantly to 27% in CRPC ($p<0.0001$). Patients with AR amplification in CRPC had a significantly shorter time to death from relapse compared to patients without AR amplification ($p=0.0002$). AR and AR213 expression is significantly higher in AR amplified tumours compared to non amplified tumours ($p=0.002$ and $p=0.025$), and AR791 expression is significantly lower in AR amplified tumours compared to non amplified tumours ($p=0.034$).
In CRPC high expression of AR and AR213 was associated of presence of metastases at relapse ($p=0.018$ and $p=0.046$, respectively) and with decreased time to death from relapse ($p=0.038$ and $p=0.003$). AR213 is also associated with a decreased disease specific survival ($p=0.008$). In contrast low expression of AR791 was associated with decreased time to death from relapse ($p=0.04$) and low expression of AR308 was associated with decreased disease specific survival ($p=0.011$) Use of paired tumours from each patient allowed changes in protein expression in the transition from hormone naive to castrate resistant disease to be assessed. Total AR, AR213 and AR650 increased with the development of castrate disease ($p<0.001$, $p<0.001$ and $p=0.003$ respectively) and AR81 decreased with the development of castrate disease ($p=0.001$).
In vitro results suggest that phosphorylation at AR213 sensitises the AR to low levels of androgens and stimulates prostate cancer growth at castrate levels. Therefore AR213 may serve as a diagnostic tool to predict patient outcome in response to maximum androgen blockade and inhibition of AR213 via the Akt pathway may be an effective therapeutic avenue to investigate for treatment of prostate cancer.

REGULATION OF ANDROGEN RECEPTOR MEDIATED TRANSCRIPTION BY RPB5 BINDING PROTEIN (RMP/URI)

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Unconventional prefoldin RPB5 Interactor (URI) was identified as a protein that binds the RPB5 subunit of polymerases and may function as a transcriptional repressor (Dorjsuren, et al., Mol. Cell Biol., 1998). URI mRNA is highly expressed in testis and our protein analysis indicates both cytoplasmic and nuclear localization in prostate cells. In addition to binding RPB5, URI was also shown to bind TFIIF and the DNA helicases TIP49 and TIP48 suggesting that URI is an important transcriptional regulator. URI also binds ART-27, an epithelial-specific AR cofactor that is decreased in prostate cancer and inhibits cell proliferation if over expressed in LNCaP prostate cells. Interestingly a gene profiling analysis of prostate cancer progression showed URI to be up-regulated in PIN (prostatic intraepithelial neoplasia) together with other Ets target genes (Tomlins et al., Science 2005), suggesting a possible role of URI in the initial stages of prostate cancer. We show that in prostate cells URI binds ART-27 and that the expression of one of the two proteins can affect the stability of the other, suggesting a tight relationship between URI and Art-27. Our data also suggest that URI as well as ART-27 is able to repress androgen receptor transcription. Knock down of URI decreases bicalutamide (casodex,CDX) inhibition of AR transcribed genes like PSA and FKBP5. Interestingly we show that in LNCaP cells, URI is phosphorylated in response to hormone (R1881) treatment and this occurs downstream of the mToR pathway. PMA, TNF-α and UV treatment of LNCaP cells also results in URI modification but these stimuli trigger URI phosphorylation downstream of p38 MAP kinase. These results suggest a role of URI in linking extracellular signaling to AR transcriptional regulation. Whole genome microarray analyzing of the impact of URI knock down on AR transcription showed that URI plays a role in the expression of genes aberrantly regulated in prostate cancer. Moreover mass spectrometry analysis of proteins interacting with URI in the nucleus of LNCaP cells showed that URI is part of several complexes involved in DNA transcription, chromatin modification, mitosis and DNA damage response. We also confirmed the binding of URI with the RPB5 subunit of RNA polymerase II as well as the binding to the general transcription factor IIF suggesting a possible role of URI in RNA polymerase II function. Moreover, LNCaP cells stably over-expressing URI protein form smaller colonies in a soft agar assay compared to control cells, suggesting that URI regulation is important for the establishment of the tumorigenic potential in prostate cancer cells. Collectively these data indicate that URI is able to represses AR-mediated gene transcription through the regulation of ART-27 co-repressor protein, RNA polymerase II transcription and possibly chromatin remodeling.

SCREENING PATIENTS WITH SEVERE INSULIN RESISTANCE IDENTIFIES A NOVEL FPLD-ASSOCIATED PPARγ MUTATION (Y151C)

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Familial partial lipodystrophy (FPLD) is considered to be a rare metabolic disorder however its clinical features may not be readily recognized. Early recognition is important because FPLD patients may require specific therapeutic approach. Here we screened medical records of patients with type 2 diabetes mellitus for extreme insulin resistance that was arbitrarily defined as the use of an insulin dosage ≥100U/day combined with a moderate to low BMI (≤ 27). From the 22 invited subjects, 12 patients were willing to participate. Five patients fulfilled the clinical features of lipodystrophy. One of these patients had previously been diagnosed as FPLD type 2, while another was known to have FPLD type 3. Three novel cases of lipodystrophy were identified in this population. One patient was a 52 year old woman with type II DM and a history of SLE and chronic corticosteroid use that could explain lipodystrophy. In one of these patients a novel heterozygous mutation (Y151C) in PPARγ was identified, located within the zinc finger structure involved in DNA binding. In vitro analysis showed impaired DNA binding capacity in the Y151C mutant and hence reduced transcriptional activity compared to wild type PPARγ. Although FPLD appears to be rare, our data indicate that severe insulin resistance as defined by low BMI and high insulin usage should trigger clinicians to perform thorough clinical examination in search of lipodystrophy.

#* authors contributed equally

LIGAND-INDEPENDENT TRANSCRIPTIONAL ACTIONS OF HUMAN ERβ SPLICE VARIANTS ON ERE, AP-1 AND HUMAN ARGININE VASOPRESSIN (HAVP) PROMOTERS IN NEURONAL CELLS

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Estrogens are critical regulators of many CNS functions including homeostasis, reproduction, learning, and memory. The actions of both endogenous and synthetic estrogens are mediated primarily by two high-affinity estrogen receptors belonging to the nuclear receptor superfamily, ERβ and ERα. The latter, and most recently identified, has several splice variant isoforms present in the brain of both rat and human. Some of the human isoforms identified to date contain variable length deletions and substitutions in exons 7 and/or 8 (e.g. ERβ1, ERβ2, ERβ4, and ERβ5), resulting in a truncated receptor protein at the C-terminus. By contrast, the rat isoforms contain deletions in exons 3 or 4 (e.g. ERβ1Δ3 and ERβ1Δ4), and also amino acid insertions in the ligand binding domain (e.g. ERβ2). Previously, we showed in neuronal cells that rat ERβ1 increased transcriptional activity mediated by an estrogen response element (ERE), and at a complex promoter for the neuropeptide arginine vasopressin (AVP). By contrast, rat ERβ1 induced a constitutive decrease in transcriptional activity mediated at the activator protein-1 (AP-1) site. Rat and human ERβ are highly homologous, therefore in this study we used the human splice variants of ERβ in order to determine the relative importance of the E and F domains for conferring ligand-independent activity when mediated by an ERE or AP-1 site, and at the human AVP promoter. In stark contrast to the rat splice variants, our results showed little to no ligand-independent activity of any human splice variant at a tandem ERE site, the exception being hERβ4, which was repressive. However, hERβ1 and hERβ4 both induced ligand-indpendent repression at an AP-1 site, similar to rat ERβ1. hERβ1 and hERβ4 also exhibit ligand-independent repression of the human AVP promoter, which was blocked with treatment of 3β-Diol, DHT, and ICI 182, 780. These data provide further understanding about the precise functions of each ERβ isoform in the brain, as well as highlight similarities and differences between the rat and human ERβ isoforms. Moreover, these data provide insight into the relative contributions of ERβ's six functional domains on mediating transcription at various cis-regulatory promoter regions.

ESTROGEN: ESTROGEN RECEPTOR ALPHA ACTIVATED NEGATIVE REGULATORS OF GROWTH FACTOR SIGNALING IN BREAST CANCER.

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Estrogen receptor alpha (ERα)-positive breast cancers co-expressing pioneer factors FOXA1 and GATA-3, which are required for efficient ERα-mediated gene expression, respond to anti-estrogen therapy. ERα:estradiol (E2) or ERα:E2-activated signaling molecules are believed to actively suppress growth factor signaling pathways in these cancers. Deregulation of this ERα function is suggested to cause aberrant activation of growth factor signaling and consequently anti-estrogen resistance. In this study, we investigated the ERα:E2 activated molecules that are likely to be involved in suppressing growth factor signaling. These studies identified TLE3, a transcription repressor, and LRIG-1, a negative regulator of growth factor receptors, as E2 inducible genes. Chromatin immunoprecipitation assays revealed binding of ERα and FOXA1 to the regulatory regions of TLE3 and LRIG-1. TLE3 is recruited to regulatory regions of target genes by FOXA1 and represses transcription. LRIG-1 is a transmembrane protein that promotes degradation of several growth factor receptors including EGFR, ERBB2, ERBB3, and Met. It appears that ERBB3 is the primary target of TLE3 and LRIG-1 as E2 mediated repression of ERBB3 transcripts correlated with binding of FOXA1 and TLE to its regulatory region. In addition, E2-mediated increase in LRIG-1 protein correlated with lower levels of ERBB3 protein. ERBB3 regulatory regions lack binding sites for ERα raising the possibility that FOXA1-TLE3 mediate E2-dependent repression of this gene. Analysis of publicly available databases showed unfavorable recurrence-free and metastasis-free survival among anti-estrogen treated patients with tumors that express lower levels of TLE3 or LRIG-1. These results suggest that ERα:E2 and FOXA1 expressing tumors with functional E2 signaling activity restrain growth factor activated signaling through TLE3 and LRIG-1. Functional studies are currently underway to determine the consequences of loss of expression of these two genes on growth factor receptor activation and anti-estrogen resistance.

DEXAMETHASONE (DEX), CORTISOL (COR), AND ALDOSTERONE (ALD) GENERATE COMMON AND UNIQUE PATTERNS OF GENE EXPRESSION IN HUMAN TRABECULAR MESHWORK CELLS

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Purpose. In addition to their well-documented ocular therapeutic effects, glucocorticoids (GCs) can cause sight-threatening side-effects including ocular hypertension and cataract. Although the molecular mechanisms of GC-induced ocular hypertension are not well-defined, GCs appear to increase aqueous humor outflow resistance and intra-ocular pressure via morphological and biochemical changes in trabecular meshwork (TM) cells. In the present study, we used Agilent microarray analysis to compare the effects of the synthetic GC DEX, the endogenous GC receptor (GR) agonist COR, and the endogenous mineralocorticoid receptor (MR) ligand ALD, on the complete transcriptome in two primary human TM cell lines. **Methods.** Transactivation of GR and MR were determined in HeLa and HEK 293T cells, respectively, by the GeneBLAzer beta-lactamase reporter gene assay (Invitrogen). For Agilent whole human genome microarray studies, TM 86 and TM 93 cells were treated with 1 μM DEX, COR, or ALD for 24 hr. Differentially expressed genes were identified using the GeneSpring analysis package, and identification of specific signaling pathways and biologic functions was performed using Ingenuity Pathway Analysis (IPA) software. **Results.** The GR transactivation EC_{50} for DEX, COR, and ALD were 2.8, 71.7, and 669.8 nM, respectively. The MR transactivation EC_{50} were 29.9, 1.6, and 0.41 nM for DEX, COR, and ALD, respectively. Microarray analysis revealed 402 and 289 genes commonly regulated by DEX, COR, and ALD in TM 86 and TM 93, respectively. These genes included RGC32, ITGA10, ANGPTL7, AOX1, FKBP5, ZBTB16, and MYOC. In addition, each agonist generated a unique set of genes in both TM cell lines. IPA analysis showed that in TM 86, ALD significantly regulated carbohydrate metabolism network, whereas DEX significantly modulated RNA-post-transcriptional modification network. In TM 93, COR significantly affected gene expression and cell cycle network, whereas DEX and COR both regulated DNA replication and repair network. **Conclusion.** The MR agonist, ALD, and the GR agonists, DEX and COR, commonly regulated a subset of genes including MYOC and FKBP5 in human TM86 and TM93 cells. More importantly, each of the three agonists was capable of generating a unique transcriptional response and presumably a unique biologic effect in these cells.

AHR-MEDIATED IMMUNOSUPPRESSION BY ALTERNATIVE AHR LIGANDS: A NOVEL STRATEGY FOR TREATMENT OF AUTOIMMUNE DISEASE

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The aryl hydrocarbon receptor (AHR) is a ligand-activated transcription factor belonging to the PER-ARNT/-SIM (PAS) family of proteins. Past research on the AHR has focused mainly on its role as the primary mediator of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity, including potent immunosuppressive effects. However, a number of recent studies suggest a potential for the AHR as a therapeutic target for the treatment of autoimmune diseases and transplantation rejection by harnessing its immunomodulatory effects. Specifically, activation of the AHR by TCDD inhibits CD4+ T cell differentiation while increasing the frequency of CD4+ CD25+ (Foxp3+/-) T regulatory cells (Tregs). These AHR-dependent Tregs promote immune suppression and ongoing tolerance of self-antigens. In support of a therapeutic potential for the AHR in the treatment of disease, treatment of the non-obese diabetic (NOD) mice model with TCDD suppresses the onset of autoimmune Type 1 diabetes, correlating with an increased population of CD4+/CD25+/Foxp3+ T regulatory cells. However, clinical use of TCDD is unlikely due to its history as an environmental toxicant. Therefore, delineation of signaling events downstream of AHR activation in T cells leading to induction of Tregs and identification of other ligands that promote AHR-dependent Tregs is crucial for advancing the potential therapeutic use of AhR ligands. Here, we present our progress in identification of such alternative ligands, several of which exhibit promise for AHR-mediated immunosuppression.

ESTROGEN RECEPTOR β IS A POTENTIAL THERAPEUTIC TARGET TO SUPPRESS LYMPHOID TUMOR GROWTH

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Estrogen receptor β (ERβ) is strongly expressed in cells of the immune system. In addition, several studies have suggested an anti-proliferative role of ERβ in normal tissues and in some cancer cells. This prompted us to analyze the effects of estradiol (E_2) and a selective ERβ agonist on lymphoma growth in culture and *in vivo*. Using immunofluorescence, we found significant nuclear expression of ERβ in murine and human Burkitt´s lymphoma cell lines. While estradiol (E_2) had only minor effects on cell growth, a selective ERβ agonist (provided by Karo Bio AB) showed a strong and significant antiproliferative effect of the murine T cell lymphoma cells and the human Burkitt lymphoma cell lines in culture. To evaluate whether natural estrogen levels may have an effect on lymphoma growth *in vivo*, male and female C57Bl/6J mice were challenged with the murine T cell lymphoma cells. Male mice developed significantly larger tumors as compared to female mice. Ovarioectomy increased tumor growth to the rate seen in males. To investigate whether lymphoma growth may be reduced by estrogen treatment *in vivo*, ovariectomized mice challenged with the murine lymphoma cells were treated with E_2 or the selective ERβ agonist. The selective ERβ agonist strongly inhibited T-cell lymphoma growth, while E_2 had a weaker inhibitory effect. The reduced tumor size seen following selective ERβ agonist treatment was mainly due to reduced proliferation but also involved increased apoptosis. Our results demonstrate an ERβ ligand-dependent antiproliferative effect of lymphoma cells expressing endogenous ERβ and that lymphoma cell growth *in vivo* can efficiently be inhibited via ERβ. This study suggests that ERβ agonists may complement currently existing therapies of lymphomas providing an inhibitory effect via ERβ on tumor cell growth.

ANDROGEN- AND GLUCOCORTICOID-INDUCED ACTIVATION OF FKBP51 LOCUS THROUGH LONG-RANGE INTERACTIONS

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FKBP51 (FK506-binding protein 51) is a sensitive biomarker of corticosteroid and androgen responsiveness in lung and prostate, respectively. We have elucidated the molecular mechanisms underlying the induction of FKBP51 by glucocorticoid receptor (GR) and androgen receptor (AR) in A549 lung and VCaP prostate cancer cells, respectively. Quantitative chromatin immunoprecipitation scans and enhancer activity analyses indicate that activation of the FKBP51 locus by glucocorticoids and androgens in vivo is triggered by the loading of their receptors to regions at ~34 kb 5’ and ~87 kb 3’ from the transcription start site (TSS), both acting as the major enhancers for the AR in VCaP cells but merely the latter one for the GR in A549 cells. Steroid exposure decreased the histone density at several regions of the gene, which was paralleled with the occupancy of SWI/SNF chromatin remodeling complexes within the locus. The proximal promoter region of the gene along with the major intronic steroid-regulated enhancer had elevated levels of H3 acetylation and H3K4 tri-methylation, whereas H3K36 tri-methylation more generally marked the gene body and reflected the occupancy of RNA polymerase II. The occurrence of these active chromatin marks within the FKBP51 locus already prior to steroid exposure suggests that it was poised for transcription in these cells. Interestingly, our ChIP scans further showed that the FKBP51 region encompassing the steroid-regulated enhancers is bordered by CTCF- and cohesin-binding (RAD21) sites in both cell lines. We propose a model in which the CTCF-cohesin complexes stabilize the chromatin loops mediating the long-range interactions between the steroid receptors and the transcription machinery.

IDENTIFICATION OF A NOVEL ESTROGEN RESPONSIVE ESTROGEN RECEPTOR A (ESR1) VARIANTS IN THE PREGNANT UTERUS.

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The role of estrogen and estrogen receptors in the onset of human labor is not clearly defined and may be involved in preterm birth. It has been postulated that increased ER action at term may inhibit uterine PR transactivation. These events would lead to a functional withdrawal of PR action and thus the onset of labor. We hypothesize that alternate ER transcripts in the pregnant uterus may play a critical role in governing PR responsiveness at term. There is mounting evidence that potential alternative ER proteins encoded by mRNA splice variants such as the hERα46 variant may play a role in the mediation of estrogen responsiveness.
Utilizing multiple ER antibodies we identified by western blot analysis two ,hERα46 and hERα51, major ER isoforms in the myocyte of human pregnant myometrial tissues, explant cultures and in our telomerase immortalized myometrial cell line (hTERT). The full length ERα66 was confined to the cytoplasmic fraction in all human uterine samples examined. To confirm the presence of the hER46/51α variant transcripts, we undertook RTPCR and RPA analysis of hTERT cell lines as well as myometrial tissues isolated from pregnant human uterus. The RTPCR and RPA analysis identified two alternate ERα transcript which when translated would give rise to a 46kDa and a 51kDa (Δ 7 isoform) ERα proteins. 5' RACE data also identified that the uterine ER46α utilized a novel promoter in intron 1 as its proximal promoter. We also determined that estrogen responsiveness of hERα46 in primary human myometrial explant cultures differ significantly between myometrium isolated from non-laboring pre-term (n=4) and term patients (n=4). Increased levels of nuclear hERα46 result upon exposure to estrogen in term patients whereas in pre-term myometrium the 46kDa isoform level remains unresponsive to estrogen. To examine if there were parallels in the mouse model system we did westerns for ERα in the mouse during pregnancy and found a truncated form of the protein in the uterus that was both in the cytoplasm as well as the nucleus but disappeared as ERα66 became the dominant form in the nucleus at term.
In conclusion we have identified in the uterine myocyte, novel ERα proteins and transcripts that utilize both novel and known promoters. We have identified in human myometrial tissues ERα46 is preferentially isolated in the nuclear fraction, hERα51 is found both in the nucleus and cytoplasm whereas the classical ERα66 is restricted to cytoplasmic fraction. Furthermore we hypothesize that the truncated forms of the estrogen receptor may as term approaches and estrogen levels rise, function as a major uterine component of estrogen action and play a role in PR functional withdrawal.

OPPOSITE ROLES OF ADDITIONAL SEX COMB-LIKE (ASXL) 1 AND 2 IN ADIPOGENESIS VIA DIFFERENTIAL REGULATION OF PPARγ

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Our previous studies indicated that ASXL1 functions as either coactivator or repressor of RAR in a cell-specific manner. In this study, we investigated the roles of ASXL family in the regulation of other nuclear receptor PPAR. GST pull-down assays showed that both ASXL1 and ASXL2, a paralog of ASXL1, interact with PPARα and PPARγ in vitro. The endogenous interaction between ASXLs and PPARγ were confirmed by immunoprecipitation (IP) assays. Transcription assays under over-expression or knockdown conditions of ASXLs indicated that ASXL1 down-regulated rosiglitazone-induced PPARγ activity, whereas ASXL2 greatly increased PPARγ activity. Similarly, ASXL1 completely repressed, but ASXL2 increased the adipogenesis as determined by Oil Red O staining using 3T3-L1 cells that stably express ASXLs. Intriguingly, ASXL1 mutant defective in heterochromatin protein 1 (HP1) binding increased the adipogenesis as much as ASXL2, indicating that HP1 binding is required for the ASXL1 repression. The adipocyte differentiation gradually reduced the expression of ASXL1 without affecting ASXL2 level. The knockdowns of ASXL1 and ASXL2 showed opposite effect on adipogenesis. Chromatin IP assays showed that HP1α and methylated H3K9 occupied the promoter of PPARγ target gene aP2 together with ASXL1 in 3T3-L1 cells. In contrast, ASXL2 occupied aP2 promoter together with acetylated H3K9, MLL1, and methylated H3K4. These occupancies were consistently changed during adipogenesis. Finally, microarray analysis demonstrated that ASXL1 repressed, but ASXL2 increased the expression of adipogenic genes, which are mostly targets of PPARγ. These results implicate that ASXL family provides diversity in the regulation of adipogenesis via differential modulation of PPARγ activity.

NHR-49 INFLUENCES MITOCHONDRIAL PHYSIOLOGY BY REGULATING DISTINCT ASPECTS OF LIPID METABOLISM

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Mammalian nuclear receptors are key regulators of mitochondrial biogenesis and energy expenditure and are therefore popular targets for promoting cardiovascular health, metabolic fitness, and overall wellbeing. In *C.elegans*, the most likely candidate for a similarly acting nuclear receptor regulating mitochondrial function is NHR-49. Like its mammalian counterparts, we show that the *C.elegans* NHR-49 targets similar metabolic pathways to modulate mitochondrial physiology in nematodes. NHR-49 orchestrates the regulation of multiple lipid metabolism pathways by partnering with distinct binding receptors to modulate a specific suite of genes. By knocking out the individual binding partner we can tease out the target genes contributing to the different *nhr-49-/-* phenotypes. One such *nhr-49-/-* phenotype is a significant alteration in intestinal mitochondrial density and morphology. The impaired expression of genes in the sphingolipid breakdown and fatty acid desaturase pathways is most likely responsible for this phenotype. Additionally we demonstrated that one of the sphingolipid breakdown genes, acid ceramidase, partially contributes to the aberrant *nhr-49-/-* mitochondrial phenotype. Our work shows the involvement of very specific genes in the nuclear receptor mediated control of mitochondrial density and shape, paving the way for a better understanding of the transcriptional regulation of mitochondrial biogenesis and function.

WARFARIN ENANTIOMERS INTERACTIONS WITH PREGNANE X NUCLEAR (PXR) RECEPTOR IN GENE REGULATION OF CYTOCHROME P450 CYP3A4 ENZYME

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Warfarin, an antagonist of vitamin K, is the oral coumarin anticoagulant widely used to control and prevent thromboembolic disorders. Warfarin is clinically available as a racemic mixture of R- and S-warfarin; S-enantiomer has 3-5 times greater anticoagulation potency than its optical congener. Recently, Vitamin K2 function has been proposed via Pregnane X receptor (PXR) in osteocytes. In the liver, PXR acts as a xenobiotic sensor that controls expression of many genes involved in drug/xenobiotic metabolism and clearance.
The aim of the paper was to examine whether enantiomers of warfarin stereoselectively interact with PXR receptor to up-regulate main drug/xenobiotic-metabolizing enzymes of cytochrome P450 superfamily. Interactions of warfarin enantiomers were tested in transient transfection gene reporter assays, TR-FRET ligand binding assay and up-regulation of PXR-target genes mRNAs has been studied employing semi-quantitative RT-PCR in primary human hepatocytes.
We found that both enantiomers interact with PXR. R-warfarin significantly induced CYP3A4 mRNA in cultures of primary human hepatocytes or in LS174T intestinal cells. S-warfarin is less potent inducer of PXR-target genes in the liver and activates PXR only in supraphysiological concentrations. We show that R-warfarin can significantly up-regulate major drug-metabolizing enzymes in the liver and thus may cause drug-drug interactions with co-administered drugs.

GLUCOCORTICOID RECEPTOR TRANSCRIPTIONAL REGULATION OF THE CIRCADIAN CLOCK GENE *PER1*

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The glucocorticoid receptor (GR) regulates numerous genes governing physiological processes including inflammation, metabolism, and development that may be influenced by the circadian clock, which itself can be entrained by GR. The molecular mechanism of this entrainment by GR has not been well described. Among the circadian genes whose cycling is affected by GR are the Period genes *PER1*, *PER2* and *PER3*. Previous work in our lab showed that GR-mediated induction of mouse *Per2* mediates glucose homeostasis in a mouse model (1), whereas GR-mediated repression of mouse *Per3* is required for the adipogenic cell fate decision (2), thus linking GR regulation of metabolism to two clock genes. To investigate the mechanism of clock entrainment, as well as gain further insight into gene-specific regulation by GR, we have begun to examine the transcriptional dynamics of human *PER1*. *PER1* encodes a clock protein essential for maintenance of the transcriptional feedback loop that constitutes the basis of the circadian program. We found that *PER1* is the circadian gene induced most rapidly and with the greatest magnitude upon addition of the GR agonist dexamethasone, and we identified two GR binding regions located near alternative transcriptional start sites. We are currently interrogating how these GR binding regions temporally operate to affect transcription from these alternative start sites, how they participate in entraining circadian rhythm, and whether GR regulation of *PER1* plays a direct role in metabolic regulation.

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LXR ACTIVATION INDUCES ARGINASE 1 EXPRESSION IN MACROPHAGES

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The ligand-activated nuclear receptors Liver X Receptors (LXRs) regulate cholesterol metabolism and immune inflammatory responses in macrophages. Moreover, activation of LXRs in macrophages inhibits the development of atherosclerosis. Enhanced levels of the hydrolytic enzyme arginase 1 (Arg1) in alternatively activated macrophages are associated with a low-atherosclerotic response in rabbits and Arg1 expression is markedly decreased in foam cells within the lesion. In humans, Arg1 SNP rs2781666 is associated with an increase in myocardial infarction. Altogether, these data suggest an atheroprotective role for this enzyme.
Arg1 expression in macrophages is largely regulated by cytokines, such as IL-4, that elicit anti-inflammatory responses. Our studies show that T0901317-activated LXRα not only induces the expression of arginase 1, but also synergistically enhances its IL-4-induced mRNA and protein expression in both the RAW 264.7 macrophage cell line and primary bone marrow-derived macrophages. In addition, a -4.8kb Arg1 promoter fragment is responsive to T0901317 in the presence of LXRα indicating that the regulation of Arg1 mediated by this nuclear receptor occurs at the transcriptional level. Our ChIP experiments, however, demonstrate that LXRα is not recruited to the Arg1 promoter in LXR ligand-treated cells. Instead, LXR appears to regulate Arg 1 expression via the increased recruitment of PU.1, an Ets transcription factor family member, to the Arg1 promoter upon LXR ligand treatment. This work provides a new molecular mechanism by which LXR agonists regulate the expression of an IL-4 modulated anti-inflammatory gene with potential implications on atherosclerosis progression.

EFFECTS OF FREE FATTY ACIDS ON THE EXPRESSION OF PGC1-α AND INSULIN SIGNALING IN SKELETAL MUSCLE CELLS

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The peroxisome proliferator activator receptor-γ coactivator 1α (PGC-1α) is a transcriptional coactivator that regulates lipid catabolism, mitochondrial number and function. Increasing evidence suggests that either reduced levels or compromised activity of PGC-1α can be associated with the development of insulin resistance (IR) and type 2 diabetes (T2D). Also, mitochondrial dysfunction and increased mitochondrial oxidative stress has been proposed to play an important role in IR and T2D. Additionally, accumulation of muscular free fatty acids (FFAs) has been implicated in IR. The type of FFAs, saturated or unsaturated, is critical in the development of IR, as the degree of saturation correlates with IR. Here, we compare the effects of the saturated FFA, palmitate, the unsaturated FFA, oleate, and a mixture of each on the production of mitochondrial reactive oxygen species (mtROS), mitochondrial function, expression of PGC-1α and mitochondrial transcription factor (TFAM), and insulin signaling pathway in rat L6 skeletal muscle cells. Only palmitate caused a significant increase of mtROS production, which correlated with concomitant mitochondrial dysfunction and inhibition of insulin signaling. Oleate alone did not cause mtROS generation and its addition prevented palmitate-induced mitochondrial dysfunction and inhibition of insulin signaling. PGC-1α protein level and promoter activity were decreased after palmitate treatment, while addition of oleate increased both PGC-1α protein level and promoter activity. Also, expression of TFAM was significantly diminished after palmitate, but not oleate, treatment. Addition of the ROS scavenger N-acetylcystein (NAC) to palmitate restored both the expression and promoter activity of PGC-1α as well as TFAM expression, suggesting that palmitate-induced oxidative stress contributed to the downregulation of these transcription factors. Based on these results we propose that ROS-induced downregulation of TFAM and PGC-1α expression may further contribute to palmitate-induced mitochondrial dysfunction and oxidative stress by decreasing mitochondrial biogenesis and thus establishing a vicious cycle of events in which palmitate-induced oxidative stress causes mitochondrial dysfunction, which causes a concomitant increase in ROS production. In this regard, the present study enhances the understanding of the mechanisms involved in IR. Identification of the pathways that regulate mitochondrial function and mtROS production can provide new potential therapeutic strategies for preventing of IR and other factors contributing to the development of T2D.

A NETWORK OF REGULATORY ELEMENTS DIRECTS THE GLUCOCORTICOID RECEPTOR TO EXPRESS PERIOD 1 AT LOW DOSES OF CORTICOSTEROID.

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As part of the human circadian cycle, cortisol acts via the glucocorticoid receptor (GR) to regulate gene expression. We previously discovered that expression of Period 1 (PER1), a key circadian regulator, is induced by 10-fold lower doses of the cortisol mimic Dexamethasone (DEX) than other GR target genes. To find additional low-dose responsive genes, we used RNA-seq to measure gene expression in A549 lung epithelial cells treated with low doses of DEX. Remarkably, we found that, in A549 cells, PER1 responds to lower doses of DEX than all other genes. To understand the mechanism of the response, we used ChIP-seq to measure genomic GR occupancy at high and low doses of DEX. Of the thousands of loci bound by GR at high DEX, only 1% are bound at a low dose concomitant with PER1 induction. We found GR occupying two sites near the PER1 transcription start site (TSS). One site, located upstream of the TSS, is bound by GR at low and high DEX, while the other site, located intronically, is only bound at high DEX. We found that the DNA sequence upstream of the PER1 TSS, including the endogenous promoter and the low-dose GR site, is sufficient to drive expression in response to low doses of both DEX and cortisol. From this larger sequence, we isolated a 250 bp enhancer surrounding the GR binding site that is sufficient for low dose expression. To test if the GR binding sequence (GBS) creates the low-dose response, we swapped the PER1 low-dose GBS with a typically responding GBS. Interestingly, we observed no change in hormone sensitivity, indicating that the context of the GBS, and not the GBS itself, is responsible for hypersensitivity of PER1 to corticosteroids. Within the low-dose PER1 enhancer, we found consensus DNA binding sites for many factors, including nuclear factor 1 (NF-1). A 2 bp mutation of one NF-1 site converted the low-dose enhancer to one with a typical response. However, introducing the NF-1 site into a typically responding enhancer was insufficient for low-dose response. Removal of other sequences in the hypersensitive enhancer also reduced dose-sensitivity, indicating that a complex interaction of cofactors is responsible for the sensitivity of PER1 expression to corticosteroids. These results show that a network of regulatory elements flanking an otherwise normal GR binding site increases sensitivity of PER1 to low levels of corticosteroids, potentially triggering production of PER1 before other genes in the circadian cycle.

AGING THE SMRT WAY: REGULATION OF MITOCHONDRIAL OXIDATIVE METABOLISM AND AGING RELATED METABOLIC DETERIORATION BY NUCLEAR RECEPTOR CO-REPRESSOR SMRT

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The transcriptional co-repressor SMRT utilizes two major receptor interacting domains (RID1 and RID2) to mediate nuclear receptor (NR) signaling through epigenetic modification. The physiological relevance of such interaction remains poorly defined. We report here a mouse model with RID1 knockin mutations, termed $SMRT^{mRID1}$, which preserves SMRT interaction with RID2 associated NRs, including peroxisome proliferator-activated receptors (PPARs). These mice exhibit reduced mitochondrial function, inhibited anti-oxidant gene expression and increased susceptibility to oxidative damage, leading to premature aging and related metabolic diseases. At the molecular level, $SMRT^{mRID1}$ suppresses PPAR activity, and the defect of $SMRT^{mRID1}$ cells in oxidative stress sensitivity is partially rescued by PPAR activation. In concert, we identify polymorphisms in the human SMRT gene associated with type 2 diabetes and adiponectin levels. These data uncover a role of SMRT RID2 in mitochondrial oxidative metabolism, which may serve as a drug target to improve health span.

INTERPLAY OF TUMOUR-DERIVED IL-6 WITH NUCLEAR RECEPTORS IN THE ACTIVATION OF BAT IN CANCER CACHEXIA/ANOREXIA SYNDROME

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Cancer cachexia involves profound metabolic imbalance leading to weight loss, muscle wasting and fat depletion. Despite the fact that cachexia is the direct cause of death in 20-30% of all cancer patients, little is known about the underlying mechanism. Multiple biological pathways may contribute to this catabolic process, including nuclear receptor signaling and pro-inflammatory cytokines, however the etiology of tissue wasting accompanied by increased metabolic rate observed in cancer patients is not known. Brown adipose tissue (BAT) plays a key role in thermogenesis & energy balance and could be responsible for physiological perturbations such as hypermetabolism. Therefore, we investigated the impact of the cachectic Colon 26 (C26) adenocarcinoma on BAT in mice.
EM and light microscopy of BAT showed profound delipidation & reduced size of brown adipocytes in cachectic C26 tumour-bearing mice, together with higher density of mitochondria. Circadian expression profiling of nuclear receptors and other molecular regulators of lipid accumulation & utilisation and their corresponding target genes showed dramatic activation of BAT. Alterations in the diurnal rhythmic expression pattern of PGC1, PPARδ, PPARγ, C/EBPα and target genes CPT1α, PBE, FAS, DGAT2, ELOV3, LPL and PERILIPIN, indicate dysregulated circadian control of lipid metabolism accompanied by increased mitochondrial and peroxisomal β-oxidation in BAT of cachectic mice. In addition, increased Deiodinase 2, Adenylate cyclase 3 and UCP1 mRNA and protein show the BAT thermogenic pathway is switched on. These changes persist when cachectic mice are acclimatized to 28oC, confirming the inappropriate activation of BAT despite thermoneutrality. While the cachectic mice do reduce food intake, pair-feeding experiments demonstrate that the changes in BAT could not be attributed to restricted nutrients. A potential link between these alterations in nuclear receptors during the development of cachexia and tumour-derived cytokines such as IL-6 is the observed increase in suppressor of cytokine signaling 3 (SOCS3) and phosphorylation of STAT3 in BAT.
CONCLUSION Chronic stimulation of IL-6 signaling in BAT of cachectic mice due to systemic release of cytokines by tumours alters nuclear receptor expression & energy homeostasis, potentially contributing to the catabolic state. Activation of BAT, despite dramatically reduced food intake, represents a maladaptive response to nutrient deprivation in cancer cachexia.

SYNERGISTIC GROWTH INHIBITION BY RETINOIC ACID AND HERCEPTIN™ IN HER2/RARA CO-AMPLIFIED BREAST CANCER CELLS

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The HER2 gene, coding for the EGFR family receptor HER2, is amplified in 25-30% of breast cancers. Herceptin, a humanized monoclonal antibody that inhibits the tumorigenic effects of HER2 is currently used for targeted therapy strategy of HER2 positive breast cancer, particularly as an adjuvant treatment strategy in combination with chemotherapy. Although initial response to Herceptin is good, the majority of patients that originally respond will develop resistance within a year. We have observed that in the HER2-positive SkBr-3 breast cancer cell line, which carries a co-amplification of the HER2 and retinoic acid receptor alpha (RARA) genes, RA and Herceptin inhibit proliferation in a synergistic way. This synergy was not observed in the HER2 amplified cell line BT-474, which does not carry the co-amplification of RARA. In addition, the observed modulation by RA in SkBr-3 cells of genes that have been proposed to be implicated in Herceptin resistance suggests that RA could help prevent the development of resistance to Herceptin. Since about 40-50% of HER2-positive tumor samples show a co-amplification of the HER2 and RARA genes, we propose that this subgroup of HER2 positive tumors could benefit from association of retinoids with Herceptin treatment.

THYROID HORMONE INDUCES THE EXPRESSION OF SERUM AND GLUCOCORTICOID REGULATED KINASE 1(SGK1) IN HEPG2 HEPATOMA CELL LINE.

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Thyroid hormone, the endocrine hormone secreted by the thyroid gland regulates crucial functions of growth, development, metabolism and homeostasis. The cellular actions of T3 are mediated by cognate thyroid hormone receptors (α and β) which are members of nuclear receptor superfamily. T3 binding to these receptors stimulates the recruitment of coactivator proteins to the thyroid hormone receptor proteins and consequent activation of downstream gene expression. Classically T3 target genes have been known to belong to groups like metabolic enzymes, transcription factors, ion channels among others. Mounting evidence indicates that T3 also causes upstream activation of certain kinase pathways in specific cell types including PI3Kinase and mTOR pathways. We have identified that T3 induces the transcriptional induction of a specific kinase called the serum and glucocorticoid regulated kinase 1 (SGK1). This protein belongs to AGC family of protein kinases and is activated downstream of PI3-kinase/Akt pathway. SGK1 has significant roles in cell survival, ion transport, salt homeostasis and integration of number of transcriptional inputs. In HepG2 cells, thyroid hormone induces the expression of SGK1mRNA and protein. At least partially, this transcriptional induction of SGK1 gene is mediated by a secondary gene product- early growth response 1 (EGR1). Thyroid hormone causes rapid induction of EGR-1 mRNA in HepG2 cells. In these cells, siRNA mediated knockdown of EGR1 led to significant reduction in the transcriptional induction of SGK1 by T3. Furthermore, EGR-1 response elements were identified in the proximal SGK1 gene promoter. Collectively our data validates SGK1 as a novel T3 target gene in HepG2 cells and identifies EGR1 as specific mechanistic pathway of SGK1 induction by T3. Given the ubiquitous nature of SGK1 expression, our findings suggest a prominent role for SGK1 in thyroid hormone function.

DUAL FUNCTION OF FOXA1 IN REGULATION OF ANDROGEN RECEPTOR BINDING TO CHROMATIN.

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Androgen receptor (AR) regulates distinct transcriptional program in a cell- and tissue-specific fashion and is of prime importance in both androgen-dependent and castration-resistant prostate cancer [1]. Our genome-wide analysis of AR-binding sites (ARBs) in LNCaP-1F5 prostate cancer cells by ChIP-seq revealed FoxA1-binding sites as the most over-represented *cis*-elements adjacent to ARBs. FoxA1 acts as a pioneer factor for ERα binding to chromatin and facilitates estrogen-dependent transcription [2,3]. However, FoxA1 is also known to recruit Groucho/TLE/Grg corepressor complexes to generate compact chromatin [4]. To understand the relationship between ARBs and FoxA1 occupancy, we used ChIP-seq to map FoxA1-binding sites on LNCaP-1F5 cell chromatin. Most of the ARBs (72%) overlapped with FoxA1-binding sites, lending credence to the role of FoxA1 as pioneer factor in androgen signaling. Interestingly, depletion of cells from FoxA1 by siRNA resulted in a dramatic increase (>2-fold) in the number of ARBs. The overlap of the ARBs in FoxA1-depleted cells to those in parental cells was only ~50%. These results imply that there are three classes of ARBs in LNCaP-1F5 cells: (i) the sites that need FoxA1 for binding to chromatin, (ii) the sites that are independent of FoxA1, and (iii) the sites the accessibility of which is masked by FoxA1 and that appear upon FoxA1 depletion. Therefore, FoxA1 appears to act both as a pioneer factor that facilitates AR binding and as a repressor that generates compact chromatin structure inaccessible to AR binding. Our expression profiling data support this conclusion, in that FoxA1 depletion both modulated the magnitude of responsiveness of a number of genes (e.g., KLK3, FKBP5, TMPRSS2, and SGK1) and permitted expression of a new set of genes upon androgen exposure. Androgen regulation of these latter genes was commensurate with the appearance of new ARBs in their regulatory regions in FoxA1-depleted cells. Collectively, the present work has revealed important new insights into the role of FoxA1 in androgen signaling and AR function.

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TRANSIENT, NON-GENOMIC ACTION OF THE GLUCOCORTICOID RECEPTOR LIMITS GAP JUNCTION INTERCELLULAR COMMUNICATION AND NEURAL PROGENITOR CELL PROLIFERATION

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Study: Glucocorticoids (GCs) are used to treat pregnant women at risk for preterm delivery; however, exposure of fetuses to GCs may trigger adverse neurological side effects mediated in part by reduced neural progenitor cell (NPC) proliferation. While many established cell cycle regulators impact NPC proliferation, other signaling molecules, such as the gap junction protein connexin 43 (Cx43), appear to also influence proliferation although its precise role and mechanism of regulation remain unresolved. Gap junction function is influenced by GCs in some cells, but hormone effects on this form of intercellular communication in NPCs and resulting functional consequences have not been examined.
Methods: NPCs from embryonic (E14) mouse cortices were subject to a 2min-1h exposure to the glucocorticoid receptor (GR) agonist dexamethasone (DEX), and/or the GR antagonist RU486 and the reversible gap junction inhibitor 1-heptanol. Cell lysates were subject to Western blot to measure Cx43 and ERK1/2 expression and phosphorylation. Gap junction intercellular communication (GJIC) was assayed by live cell Fluorescence Recovery after Photobleaching (FRAP). Cell proliferation was measured by assessing S phase (i.e. BrdU incorporation) and cell cycle (i.e. Ki67 expression) progression.
Results: Treatment of NPCs with Dex for 1h led to both significantly increased Cx43 phosphorylation at Ser279/282, an ERK target site, and decreased GJIC. All DEX effects were blocked by continuous co-administration of RU486. ERK1/2 activation (as measured by Western blot analysis of ERK1/2 phosphorylation) was transient and first detected within 2min of DEX treatment. A 1 hr delay in the addition of RU486 following DEX exposure led to a significant reduction in cell proliferation by 24h, as did 1h 1-heptanol treatment. Pre-treatment with the transcription inhibitor Actinomycin D did not reverse the decrease in GJIC following 1H DEX treatment. Co-treatment with the ERK inhibitor PD98059 reversed the DEX induced increase in Cx43 phosphorylation and the loss in GJIC.
Conclusion: These findings indicate unique, rapid, non-genomic and MAPK-ERK1/2 dependent effects of GCs on regulation of GJIC and subsequent proliferation in NPCs. Strikingly, a 1h delay in the addition of RU486 following DEX did not reverse the DEX effects on cell proliferation, suggesting that rapid actions of GR are responsible for DEX-dependent inhibition of NPC proliferation.

THE GENE REGULATORY RESPONSE OF MOUSE AORTIC CELLS TO ESTROGEN IS LINKED TO A DISTINCT DISTRIBUTION OF ERα ON CHROMATIN.

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Studies over the last decade indicate that estrogen protects pre-menopausal women against cardiovascular disease, at least in part, by binding to and activating the estrogen receptors (ERs), ERα and ERβ. However the idea of using estrogen to protect against cardiovascular disease (or, indeed, the use of estrogen as part of hormone therapy to treat adverse menopausal symptoms) is greatly complicated by the fact that estrogen treatment has different effects in different tissues. In particular, estrogen treatment increases the risks of venous thrombosis and of cancer in the uterus and breast. The effects of estrogen are largely mediated by the transcriptional regulatory response that occurs when estrogen-bound ERs are recruited to specific chromatin locations. Importantly, recent studies have shown that the gene regulatory effects of the active, natural form of estrogen (17β estradiol, E2) differ greatly between human MCF7 and U20S cell lines, as do the chromatin binding sites for ERα. These results suggest that tissue-specific responses to estrogen are mediated by tissue-specific distributions of ER, and highlight the importance of performing mechanistic studies in vascular tissues for understanding the vasoprotective effects of estrogen.

Studies from our research group have shown that the genomewide gene regulatory response of mouse aortic tissue to E2 changes dramatically with time of hormone treatment (2, 4 or 8 hrs). To begin to understand the mechanisms for this response, we recently mapped the genomewide chromatin locations of ERα in mouse aorta after 45 minute E2 treatment, using ChIP-seq. Our results indicate that ERα binding within 50kb of transcription start sites is strongly associated with transcriptional upregulation, but not with downregulation. Furthermore, the fraction of upregulated genes with nearby ERα binding peaks is higher at early time points than at 8 hrs, consistent with the model that early regulated gene products contribute to the long term response to E2. Comparing our results to those from a recent study in mouse liver tissue, we find very little overlap between regulated genes and ERα binding sites in aorta versus liver, consistent with the hypothesis that the response of aortic tissues to E2 is largely determined by an aorta-specific distribution of ER. The results of these studies are helping to refine new mechanistic models for vascular-specific gene regulatory responses to estrogen.

THE TESTOSTERONE METABOLITE 5α-ANDROSTANE-3β, 17β-DIOL (3β-DIOL) LEADS TO DIFFERENTIAL ERα AND β OCCUPANCY OF THE OXYTOCIN (OT) PROMOTER, A STRESS-RELATED GENE.

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Although best known for its female reproductive functions, oxytocin (OT) also participates in regulation of the stress response in both males and females. It is expressed in hypothalamic neurons that participate in the stress response and is regulated by the predominant circulating sex steroid. The mechanisms of OT regulation by testosterone, in particular, are poorly understood. Recently it has been found that 3β-diol, a testosterone metabolite, binds estrogen receptor beta (ERβ). Thus, circulating testosterone may regulate OT expression by its conversion to 3β-diol, which then binds to ERβ. In the present study a hypothalamic cell line is being used to compare the effects of E2 and 3β-diol on occupancy of the OT promoter by ERα/β and co-regulators. The type and degree of histone modifications elicited are being monitored, as well. ChIP analysis has been targeted to a region of the OT gene promoter that contains a composite estrogen response element (ERE). At 30 mins, E2 failed to alter the occupancy of OT both by ERα or ERβ. It also failed to alter the degree of H3 or 4 pan-Acetylation. At 60 mins, however, E2 down regulated all these parameters: ERα & β by 40% and pan-AcH3 and 4 by 60%. In contrast to E2, at 30 minutes 3β-diol increased ERβ occupancy 3 fold and the level of pan-acetylated H4 (pan-AcH4) 4 fold. There was no effect on ERα occupancy or the degree of pan-AcH3. At 60 mins, however, 3β-diol decreased ERα and β occupancy to the same levels as E2 had, but had no significant effect on the pan-acetylation of either histone. Thus, the two ligands lead to different patterns of occupancy by ERα and β receptors and to different levels of H3 and 4 pan-acetylation. The 3β-diol effect corroborates the hypothesis that testosterone regulates OT expression via this metabolite. Furthermore, they suggest that this stress-related gene is regulated by ERα and β both in females and males and does so via different kinetics. Funded by NS039951 (RJH; RMU co-investigator)

INTEGRATION OF IL-1β AND ANDROGEN RECEPTOR SIGNALING: CHARACTERIZING TWO DISTINCT INTERSECTIONS

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Inflammation plays a critical role in the body's ability to maintain and protect itself from invading pathogens. However, chronic onslaught and over-activity can both change the signaling pathways and mark the genes that respond to inflammatory signaling. Chronic inflammation is a well-characterized contributor to diseases such as diabetes, atherosclerosis and arthritis and there have been numerous studies linking chronic inflammation and infection with prostate cancer. IL-1β is a pro-inflammatory cytokine that activates the JNK and p38 stress kinase pathways as well as NF-κB. Infiltrating inflammatory cells, such as macrophages and monocytes, release inflammatory cytokines and are found in malignant tissues, such as prostate cancer. Additionally, primary prostate epithelial cells have been shown to secrete IL-1β at the picogram per milliliter level.

IL-1β affects the androgen receptor in two rather disparate ways. First, it modulates AR directly through phosphorylation at S650, which impacts AR mediated transcription at specific genes. Additionally 40% of prostate cancer samples investigated showed robust S650 staining in the nucleus indicating phosphorylation at S650 may play a role in a distinct subset of cancers. Second, activation of NF-κB suppresses transcription of AR through binding of MybB and p65 at the AR promoter. This decreases the levels of AR in LNCaP cells over multiple passages slowing their growth. Over time, however, AR levels are reestablished. Initially IL-1β signaling is protective, in the sense that it completely blocks anchorage independent growth, a hallmark of cellular transformation. Cells that have been acclimatized to IL-1β, however, are able to grow in anchorage independent conditions regardless of further IL-1β treatment. This suggests chronic inflammation and acute inflammation have very distinct effects on prostate cancer cells. We have established a model system to investigate these differential effects on growth and cell signaling by culturing LNCaP cells long-term in IL-1β.

ENTERIC BACTERIAL REGULATION OF VITAMIN D RECEPTOR SIGNALING IN INTESTINAL INFLAMMATION

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Vitamin D receptor (VDR) is a nuclear receptor that mediates most known functions of 1,25-Dihydroxyvitamin D (1,25(OH)2D3), the hormonal form of vitamin D. VDR and 1,25(OH)2D3 are involved in calcium homeostasis, electrolyte and blood pressure regulation, immune response, and anti-inflammation activity. The target genes of the VDR signal pathway include the enzyme Cyp24 and antimicrobial peptides beta-defensin and cathelicidin. Not surprisingly, VDR plays an essential role in gastrointestinal inflammation. Polymorphisms in VDR gene are associated with susceptibility to inflammatory bowel disease (IBD). Moreover, VDR deficient mice are more susceptible to bacterial infection. However, the majority of studies of vitamin D, VDR, and inflammation are focused on immunoregulation, with little emphasis on assessing the effects of VDR in intestinal epithelial cells. It is unknown how intestinal VDR signaling responds to commensal and pathogenic bacterial stimulation. This current study investigated the effects and mechanism of pathogenic enteric bacteria on VDR expression. We hypothesize that VDR expression determines how intestinal epithelial cells respond to pathogenic bacterial triggers. We found that mice lacking VDR had high amount of serum inflammatory cytokines and worse outcomes following *Salmonella*-induced infection. Moreover, we found that enteric bacterial colonization directly regulated VDR expression, distribution, and activity in intestinal cells. Furthermore, we defined the molecular mechanism of VDR negatively regulating the intestinal NF-kappaB activity in the pathogenic bacteria stimulated cells. We showed that VDR negatively regulated bacterial-stimulated intestinal epithelial NF-kappaB activity. Our studies provide new insight into the anti-inflammatory mechanism of VDR in inhibiting intestinal infection. Endogenous enteric bacteria play a crucial role in the pathogenesis of IBD. Our studies will establish a new target—VDR signaling for treatment of inflammatory bowel disease.

PURIFICATION AND IDENTIFICATION OF RORγT CO-REGULATORS IN T HELPER CELLS

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The differentiation of interleukin 17 (IL-17)-producing T helper cells (Th17 cells) are important to the pathogenesis of autoimmune inflammation. Retinoic acid receptor-related orphan receptor gammat (RORγt) is a master regulator for Th17 cell differentiation. However, molecular mechanism of RORγt-dependent transcriptional control remains unclear. Here, we established large scaled culture system and a purification system of RORγt associating proteins from T cell 68-41 hybridoma cells. Associated proteins were purified with endogenous anti-RORγt antibody column and separated by SDS-PAGE, then identified by MALDI-TOF/MS. Besides known nuclear receptor-related co-regulators, novel transcriptional co-regulators of RORγt are identified. We found that these proteins act as transcriptional co-activators for RORγt by using luciferase reporter assay. Interestingly, some proteins functioned as transcriptional co-regulators for Smad, NFAT, and AP1. Identified proteins regulated endogenous mRNA expression levels of IL17 induced by T cell receptor activation, TGFβ, and IL6 in 68-41 cells and primary helper T cells. Furthermore, overexpression of identified proteins changed the recruitment pattern of histone H3K4me2 on IL17 promoter region.
Thus, in this study we demonstrated the existence of transcriptional co-regulators supporting RORγt function in helper T cells.

IDENTIFICATION AND FUNCTIONAL ANALYSIS OF AP2γ AS A NOVEL TRANSCRIPTIONAL COFACTOR OF ESTROGEN RECEPTOR IN BREAST CANCER

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Estrogen receptor (ER) is important in the development and progression of breast cancer. Using ChIP-sequencing, we mapped the genomic landscape of ERα binding sites (ERBS) in the breast cancer cell line, MCF-7. To understand what factors can influence the transcriptional activity of ERα, we performed cofactor motif analysis on the ERα binding sites identified by ChIP-sequencing. Our analysis revealed the binding sequences of AP2 family of transcription factors were highly enriched in the ERBS. The AP2 family of transcription factors is known to orchestrate a variety of cellular processes, including cell growth, cell adhesion and tissue differentiation. AP2γ, a member of the AP2 family, has been shown to be over-expressed in breast tumors, however little is known on how it regulates transcription in breast cancer. Here, we showed that AP2γ is recruited to the distal ERBS of the estrogen regulated gene, rearranged during transfection (RET), in a ligand-independent manner. Using a combination of siRNA, ChIP and Chromosome conformation capture (3C) assays, we showed that AP2γ is essential for the recruitment of ERα and FoxA1, and the long-range chromatin interaction at the RET gene. Moreover, down-regulation of AP2γ resulted in reduced RET transcription. Taken together, our data suggests that multiple transcription factors (e.g. AP2γ and FoxA1) are pre-loaded at ERBS establishing functional complexes of cofactors that are required for the coordinated regulation of ERα activity.

CANCER CACHEXIA SYNDROME: IMPACT OF TUMOUR-DERIVED IL-6 ON NUCLEAR RECEPTORS AND CIRCADIAN REGULATION OF METABOLIC PATHWAYS IN LIVERS OF CACHECTIC MICE

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Cancer cachexia is a catabolic condition characterized by progressive weight loss, fat depletion & muscle wasting. The impact of cachexia is severe, occurring in 20-80% of cancer patients depending on the tumour type and the direct cause of death in ~30% of all cancer sufferers. Although the mechanism of cachexia is unknown, there is a strong evidence for the involvement of increased systemic levels of tumor-derived cytokines (eg IL-6). Our focus has been primarily on the liver and hepatic metabolic pathways fundamental for the control of total body homeostasis. Given the integral role of nuclear receptors in regulating metabolic patways linked with circadian rhythm, we characterized the diurnal expression pattern of nuclear receptors & genes they regulate in the livers of cachectic colon 26 (C26) tumour-bearing mice. Expression profiling was carried by cDNA microarray and MS-based iTRAQ proteomics. Six-timepoint analysis by RT-qPCR of the core circadian clock genes - BMAL1, CLOCK, CRY1 and PER2 as well as the nuclear receptors REVERBα and RORα showed significant changes to their normal rhythmic diurnal patterns, particularly at 2am and 2pm. Analysis of liver samples by cDNA microarrays identified numerous genes which displayed a rhythmic pattern in normal mice (ie peaking at 2am or 2pm) many of which lost the normal circadian expression pattern in cachectic C26 livers. PPARα/δ/γ as well as ERRα all displayed markedly reduced expression, along with their target genes in fatty acid β-oxidation (PBE, HADHA & B), fatty acid uptake (LPL), lipogenesis (FAS, SCD1) and glucose/pyruvate metabolism (PEPCK, PDK4). Expression profiling demonstrated decreased expression of many other genes& proteins in key energy generation pathways which is counter-intuitive to the expected role of the liver in settings of food restriction/weight-loss - ie to adaptively switch on fatty-acid use, ketone body production & gluconeogenesis. Evidence for links between such widespread disruptions to hepatic metabolic pathways & elevated tumour-derived IL-6 include activated STAT3 & increased SOCS3 mRNA and hepatic acute-phase proteins. Therefore distal tumours drive chronic stimulation of cytokine-signaling in the liver altering the action of nuclear receptors and hence disrupting metabolic pathways responsible for maintaining energy homeostasis. The net outcome of impaired processing & supply of nutrients to muscle, fat & other organs would contribute to the devastating effects of cachexia.

PPARGAMMA IN REGULATION OF AORTIC STIFFNESS AND PULSE PRESSURE

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Loss-of-function mutations in PPARG gene are implicated in human hypertension and PPARgamma agonists decrease blood pressure both in animal models and human patients. How PPARgamma affects blood pressure and vascular reactivity is still unclear in vivo. To study the role of PPARgamma in the pathogenesis of hypertension and vascular diseases, we generated mice with extremely low PPARgamma expression. Interestingly, we found that PPARgamma deficiency induced an increase in systolic blood pressure without significant change in diastolic blood pressure, ultimately causing a significant increase in pulse pressure. Loss of aortic elasticity, generally termed aortic stiffness, is thought to be one of the most important factors contributing to the increased pulse pressure. Thus, we hypothesized that PPARgamma deficiency impairs aortic elasticity, resulting in the increase in pulse pressure. Aortic pulse wave velocity (PWV) was significantly increased in PPARgamma hypomorphic mice, suggesting that aortas of PPARgamma hypomorphic mice were stiffer than those of wild-type mice. Expression of elastin in aortas was significantly decreased, and negatively correlated with aortic PWV in PPARgamma hypomorphic mice. This was accompanied by increased expressions in gene and protein of MMP-9 in aortas of PPARgamma hypomorphic mice. Together, our results suggest that PPARgamma deficiency impairs elasticity in aorta and underscore the importance of PPARgamma in the regulation of blood pressure and vascular reactivity.

FOXL2 INTERACTS WITH ER IN FEMALE SEX MAINTENANCE

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Foxl2 is a forkhead transcription factor essential for proper reproductive function in females. Human patients carrying mutations in the *FOXL2* gene display Blepharophimosis/ Ptosis/Epicanthus inversus Syndrome (BPES), an autosomal dominant disease associated with eyelid defects and premature ovarian failure in females. Likewise, mice homozygous mutant for *Foxl2* display craniofacial malformations and female infertility. To elucidate the molecular mechanisms underlying the impaired folliculogenesis of *Foxl2* knockout mice, we have deleted the *Foxl2* gene postnatally using the Cre-loxP system. Surprisingly, several male-specific genes reported to be essential for testis development are upregulated in *Foxl2* mutant ovaries. In males, the transcription factor SRY, encoded by the Y chromosome, is normally responsible for triggering the indifferent gonads to develop as testes rather than ovaries. Inducible deletion of Foxl2 in adult ovarian follicles leads to immediate upregulation of testis-specific genes including the critical SRY target gene *Sox9*. The cell fate switch of ovarian granulosa into testicular Sertoli cells is accompanied by transdifferentation of theca cell lineages into Leydig-like cells with ectopic expression of the testosterone-synthesizing enzyme 17ßHSD3 and elevated serum testosterone levels comparable to male animals. A similar phenotype of postnatal sex reversal has been observed in ovaries of Estrogen Receptor (ER) alpha beta double mutant mice. Using various approaches such as immunoprecipitation, luciferase assays and transgenic mouse lines, we show that Foxl2 interacts with ER both physically and genetically to repress expression of *Sox9* from a gonadal-specific cis regulatory element. Our results show that maintenance of the ovarian phenotype is an active process throughout life. They might also have important medical implications for the understanding and treatment of some disorders of sexual development in children and premature menopause in women.

GENOME WIDE ANALYSIS OF VDR BINDING SITES IN HUMAN MONOCYTES

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The effects of the biologically most active form of vitamin D, 1α,25-dihydroxyvitamin D3 (1α,25(OH)2D3) are mediated through the vitamin D receptor (VDR), which is a member of a superfamily of ligand-inducible nuclear receptors that control expression of their primary target genes in response to binding of steroidal or other lipophilic compounds. VDR binds as a heterodimer with retinoid X receptor (RXR) to specific segments of DNA, the vitamin D response elements (VDREs). In order to locate genome wide the VDREs we used an in silico screening method, which was based on systematic EMSA assays of VDR binding to a large panel of DR3 type VDREs. The in silico screening data was challenged using FAIRE and ChIP -seq using antibodies against VDR and RXR in the presence and absence of 1α,25(OH)2D3. Finally, we used microarrays to determine the genes that were affected by the 1α,25(OH)2D3 treatment.

PPARγ INHIBITS ALLOGENIC CTL CYTOTOXICITY

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Cytotoxic T cells (CTL) are important mediators of the adaptive immunity organizing the defence against virus-infected or tumour cells. Moreover, during transplantation, CTL can contribute to pathological conditions, such as acute organ rejection. Therefore, CTL inhibition is a desired therapeutic approach. Taking into consideration that PPARγ has been established to attenuate $CD4^+$ T cell activation, we elucidated whether PPARγ can alter CTL activity as well. Thus, we established a non-radioactive allogenic in vitro cytotoxicity assay. Our experimental set up included P815 (haplotype $H\text{-}2^d$) cells as target cells and CTL derived from spleens of C57BL/6 mice (haplotype $H\text{-}2^b$) as effector cells. To initially activate effector cells, CTL were co-incubated with γ-irradiated splenocytes derived from Balb/c mice (haplotype $H\text{-}2^d$) for 10 days. Following this first step, effector cells were co-incubated with P815 target cells, beforehand stained with CellTrackerOrange to follow cell demise. CTL-dependent target cell lysis was analyzed after 1 and 24 h by flow cytometry. Although 1 h after CTL addition no target cell destruction was detectable, the amount of living CellTrackerOrange positive target cells was decreased to approximately 50% after 24 h. The optimal target vs. effector cell ratio was 1:10. Raising the ratio to 1:50 did not enhance CTL-dependent cytotoxicity, whereas decreasing it to 1:5 or lower, significantly increased the number of surviving target cells. Incubating CTL for 1 h with 1 μM of the PPARγ-specific agonist rosiglitazone diminished target cell lysis to 25%. To verify a PPARγ-dependent effect, CTL were derived from T-cell specific conditional PPARγ knockout ($LckCre^{+/-}PPAR\gamma^{fl/fl}$) mice. As to our expectation, CTL not expressing a functional PPARγ the addition of 1 μM rosiglitazone did not alter CTL-dependent cytotototoxicity. To characterize the molecular mechanism how PPARγ inhibits cytotoxicity, we analyzed the expression of genes established in mediating CTL-dependent cytotoxicity (FasL, perforin and granzyme B) by quantitative PCR in wild type and conditional PPARγ knockout mice. We found that FasL and granzyme B expression are increased in response to allogenic target cell addition, whereas perforin expression was not induced. In PPARγ wild type CTL the induction of FasL and granzyme B expression was attenuated in response to rosiglitazone treatment. In agreement to the observation in the cytotoxicity assay, rosiglitazone did not alter FasL and granzyme B expression in CTL not expressing a functional PPARγ. From these data we conclude that PPARγ mediated inhibition of FasL and granzyme B expression contributes to attenuate CTL-dependent cytotoxicity. Our data suggest that PPARγ-dependent inhibition of CTL cytotoxicity might be a new therapeutic approach to reduce transplant rejection.

DOSE-DEPENDENT EFFECTS OF PROGESTINS ON PROGESTERONE RECEPTOR ACTIVITY AND TARGET GENE EXPRESSION: LESS IS MORE?

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While the molecular mechanisms by which the progesterone receptor (PR) regulates breast tumor growth and metastasis have not been fully elucidated, recent studies highlight the fact that PR has a complex role in breast cancer progression that may be dependent on ligand dose. For example, data from the Women's Health Initiative trial suggest that low-dose progestins used in combination with estrogen are associated with an increased risk of invasive breast cancer compared to estrogen alone. On the other hand, high-dose progestin therapy has been used as an effective front-line and second-line treatment for advanced, metastatic breast cancer. To investigate this paradox, we undertook studies to identify aspects of PR biology that respond differently to treatment with low or high doses of progestins. Interestingly, we found that treatment of T47D breast cancer cells with low-dose progestins generates a significantly more robust induction of a subset of PR target genes, including the cell cycle regulators cyclin D1 and E2F1, than treatment with high-dose progestins. Furthermore, we show that varying doses of progestins have differential effects on the receptor itself, including the subcellular localization, phosphorylation and subsequent turnover of PR. At the level of transcriptional regulation, our ChIP analyses have led us to hypothesize that the strength of PR activation of target gene expression may hinge upon the ratio of phospho-PR versus unmodified PR that is recruited to enhancer elements. This dose-dependent effect may result in the formation of different PR transcriptional complexes and explain the diverse downstream biologies that are regulated by varying levels of progestins throughout the female reproductive cycle and in diseases such as breast cancer. Given the wide range of medical therapies that utilize progestins, our findings may have important clinical implications.

RAC3 IS A NOVEL PRO-MIGRATORY COACTIVATOR OF ERα WITH LINKS TO BREAST CANCER PROGRESSION

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ERα is a ligand-dependent nuclear receptor that is important in breast cancer genesis, behavior and response to hormone-based therapies. T7 phage display screening coupled with human genome-wide exon arrays was used to identify RAC3 as a putative ERα coregulator. RAC3 is a rho family small GTPase that is overexpressed in breast cancer, but its role in the cell is best characterized in the cytoplasm and membrane, and associated with cytoskeletal rearrangement. Here we show a novel role for nuclear RAC3 as an ERα transcriptional activator with implications for metastatic disease. Through in vitro and cellular work, RAC3 was shown to exist in a GTP-bound state and act as a ligand specific ERα coactivator of E2 induced transcription. Overexpression of RAC3 induced pro-growth and pro-migratory genes that resulted in increased migration in breast cancer cells. Chemical inhibition and genetic knockdown of RAC3 antagonized E2 induced cell proliferation, cell migration, and ERα mediated gene expression, indicating that RAC3 is necessary for full activity of ERα. In agreement with the molecular data, RAC3 overexpression in ERα positive breast cancer correlated with a significant decrease in recurrence and a significant increase in the odds ratio of metastasis. In conclusion, RAC3 is novel coactivator that promotes cell migration and has clinical implications for ERα positive breast cancer metastasis. RAC3 may be a useful drug target for ERα+ breast tumors that fail antiestrogen or aromatase therapy.

SPECIFIC PHOSPHORYLATION OF COACTIVATOR MEDIATOR 1 CODING FOR UBE2C LOCUS LOOPING LEADS TO ANDROGEN RECEPTOR NEGATIVE PROSTATE CANCER GROWTH

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The UBE2C oncogene is overexpressed in many types of solid tumors including the lethal castration-resistant prostate cancer (CRPC) that is highly heterogeneous in androgen receptor (AR) expression. While we have identified two CRPC-specific AR-bound enhancers that drive UBE2C overexpression in AR positive CRPC, little is known about the regulation of UBE2C in AR negative CRPC. Here we used a locus-centric chromosome conformation capture approach to identify three AR negative CRPC-specific enhancers. We further show that selective phosphorylation of coactivator Mediator 1 (MED1) in AR negative CRPC is required for these enhancers to interact with the UBE2C promoter through chromatin looping, leading to AR negative CRPC growth. Our results not only suggest that development of specific therapies for CRPC should take account of targeting MED1 phosphorylation, but also define a novel role for the Mediator complex in forming or sustaining active chromatin structure via MED1 phosphorylation.

THE REDUCTION OF LXR ACTIVITY BY EZETIMIBE IMPROVES THE FATTY LIVER AND DIABETES.

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Ezetimibe is the inhibitor of cholesterol absorption and used in treating hyperlipidemia. It has been reported that ezetimibe not only decreases serum total cholesterol, but also reduces serum triglyceride and improves hepatic steatosis and insulin resistance in mice and human. However, that mechanism is not fully understood. Here we report the mechanism for that. We used KK-Ay mice, which is the animal model of the diabetes and obesity, fed a high fat diet with or without ezetimibe. In ezetimibe-treated mice, serum and hepatic lipids, serum glucose and insulin were decreased and the hepatic steatosis was ameliorated. OGTT and IPITT indicated improved glucose tolerance and insulin sensitivity. Administration of ezetimibe decreased liver X receptor (LXR) activity and lipogenic gene expressions in liver. Simultaneously, reactive oxygen species (ROS) and inflammatory cytokines were diminished. Consistent with that, in liver, JNK phosphorylation and IRS serine phosphorylation were decreased. These observations suggest that ezetimibe causes the reduce of LXR activity, followed by hepatic lipogenic gene expressions, and thereby decreases ROS production, and so reduces JNK phosphorylation and then improves insulin resistance.

TISSUE-SPECIFIC IN VIVO ROLES OF NUCLEAR RECEPTOR COFACTOR MED1

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Mediator recently has emerged as a central player in the direct transduction of signals from transcription factors to the general transcriptional machinery. MED1 (a.k.a. TRAP220/DRIP205/MED220) subunit of this complex has been shown to directly interact and mediate the transcription of nuclear receptors, in a ligand-dependent fashion, through its two classical LxxLL motifs. However, despite the strong in vitro evidence, there currently is little information regarding in vivo functions of the LxxLL motifs either in MED1 or in other coactivators. Towards this end, we have generated MED1 LxxLL motif-mutant knockin mice. Interestingly, these mice are both viable and fertile, and do not exhibit any apparent gross abnormalities. However, they do exhibit severe defects in pubertal mammary gland development. Consistent with this phenotype, as well as loss of the strong ligand-dependent estrogen receptor (ER)α-Mediator interaction, expression of a number of known ERα-regulated genes was down-regulated in MED1-mutant mammary epithelial cells and could no longer respond to estrogen stimulation. Interestingly, our experiments showed that estrogen-stimulated mammary duct growth but not uterine development was greatly diminished in of MED1-mutant mice. Furthermore, we found that MED1 is differentially expressed in different types of mammary epithelial cells and that its LxxLL motifs play a role in mammary luminal epithelial cell differentiation and progenitor/stem cell determination. These results, together with our most recent finding that muscle specific deletion of MED1 enhances insulin sensitivity, glucose tolerance and resistance to high fat diet-induced obesity, establish key tissue- and nuclear receptor- specific roles for MED1 in vivo.

IDENTIFICATION AND EXPLOITATION OF TARGETABLE PROTEINS/PROCESSES IN THE AR SIGNALING PATHWAY INVOLVED IN PROSTATE CANCER PATHOGENESIS

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Significant progress has been made in defining the molecular mechanism(s) by which cells distinguish between AR agonists and antagonists and how some receptor modulators can manifest their actions in a cell-selective manner. The most important of these are (1) differences in the relative expression level of receptor subtypes or isoforms, (2) the impact which the bound ligand has on the structure of its cognate receptor, and (3) the complement of coactivators and corepressors in a target cell which can interact with the activated receptor. These advances in our understanding of the molecular pharmacology of nuclear receptors have provided insights into the processes underlying drug resistance to antihormonal therapies and have provided direction to efforts aimed at the development of improved cancer therapeutics. We have developed and applied a cofactor interaction/AR conformation-based screen to define important mechanistic distinctions between existing antiandrogens and to direct the discovery of new classes of antagonists that function by unique mechanisms and which exhibit favorable activities in cellular models of hormone refractory prostate cancer. These findings highlight the primacy of receptor conformation in determining the pharmacological activities of bound ligands and provide direction for the pharmaceutical exploitation of this receptor in prostate cancer and other androgenopathies.

In addition to focusing on AR itself we have also embarked on efforts aimed at identifying druggable targets downstream of AR that may yield useful therapeutics. Among the targets identified in this manner was a functionally active splice variant of the serine/threonine kinase *Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ)*. Importantly, this variant was highly expressed in both the prostate and in prostate cancers. Inhibition of the CaMKKβ activity using pharmacological inhibitors or siRNA-mediated knockdown blocks androgen-mediated migration and invasion. Conversely, overexpression of the CaMKKβ variant alone led to increased migration. Given the key roles of CaMKKβ in the biology of prostate cancer cells, we propose that this enzyme is a potential therapeutic target in prostate cancer.

Taken together the results of these studies highlight the utility of applying advances in our understanding of the molecular pharmacology of a well-validated target (AR) to identify useful prostate cancer therapeutics.

CONSTITUTIVELY ACTIVE ANDROGEN RECEPTOR SPLICE VARIANTS EXPRESSED IN CASTRATION-RESISTANT PROSTATE CANCER REQUIRE FULL-LENGTH ANDROGEN RECEPTOR

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Androgen receptor (AR) splice variants lacking the ligand binding domain (ARVs), originally isolated from prostate cancer cell lines derived from a single patient, are detected in normal and malignant human prostate tissue with the highest levels observed in late stage, castration-resistant prostate cancer. The most studied variant (called AR-V7 or AR3) activates AR reporter genes in the absence of ligand and could therefore play a role in castration resistance. To explore the range of potential ARVs, we screened additional human and murine prostate cancer models using conventional and next generation sequencing technologies and discovered several new, structurally diverse AR isoforms. Some, like AR-V7/AR3, display gain-of-function whereas others have dominant interfering activity. We also find that ARV expression is coupled to full-length AR (AR-FL) mRNA production, both of which increase acutely in response to androgen withdrawal and are suppressed by testosterone. As expected, constitutively active, ligand-independent ARVs such as AR-V7/AR3 are sufficient to confer anchorage-independent (in vitro) and castration-resistant (in vivo) growth. Surprisingly, this growth is blocked by ligand binding domain targeted antiandrogens such as MDV3100 or by selective siRNA silencing of AR-FL, indicating that the growth promoting effects of ARVs are mediated through AR-FL. These data suggest that the increase in ARV expression in castrate-resistant prostate cancer is an acute response to castration rather than clonal expansion of castration or antiandrogen-resistant cells expressing gain-of-function ARVs and furthermore provide a strategy to overcome ARV function in the clinic.

GROWTH REGULATION BY ANDROGEN RECEPTOR IN ESTROGEN RECEPTOR-NEGATIVE BREAST CANCER

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Estrogen receptor (ER)-negative breast cancers account for 25-30% of all cases and derive little or no benefit from current endocrine therapies. We have investigated the potential of targeting the androgen receptor (AR) in ER- breast cancers. AR is expressed in 60-70% of human breast tumors, independent of the ER status. Interestingly while androgens inhibit the growth of ER+/AR+ breast cancer cells, they stimulate the growth of those that are ER-/AR+. The mechanism, however, by which androgens and AR function to regulate breast cancer growth remains largely unknown. Analysis of gene expression profiles from human breast tumors revealed enriched expression of AR and androgen-regulated genes in the ER- breast tumors that over-express HER2. Through the analysis of the AR cistrome and androgen-regulated gene expression profiles in ER-/HER2+ breast cancer cells we find that AR mediates ligand-dependent activation of Wnt/β-catenin and HER2 signaling pathways through direct transcriptional up-regulation of WNT7B and HER3. Specific targeting of AR, Wnt/β-catenin or HER2 impairs androgen-stimulated tumor cell growth. Thus, these findings suggest a potential role for therapies that target AR in ER-/HER2+ breast cancers.

AN EPIGENETIC CELL-BASED SCREEN IDENTIFIES A NOVEL SMALL MOLECULE WITH SPECIFIC ANTI-CANCER ACTIVITY WHICH MODULATES TRANSCRIPTION

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Epigenetic pathways are often aberrant in cancer cells, altering transcriptional control. Examples include the overexpression or mistargeting of DNA methyltransferases leading to silencing of tumor suppressor gene promoters, mutations in histone acetyltransferases resulting in their inactivation, or the abnormal recruitment of histone deacetylases to promoter regions by oncogenic fusion proteins, consequently inhibiting the expression of differentiation genes. Some of these aberrant epigenetic events can be reversed by modulating the action of epigenetic enzymes with chemical inhibitors, yet few inhibitors are available. The recent identification and characterization of an increasing number of histone modifying enzymes involved in epigenetic events makes it more imperative to develop chemical tools to modulate their biological activity. We have developed a unique cell-based system to screen for small molecule modulators of epigenetic pathways. This system has allowed us to identify candidate compounds that have the ability to re-activate the expression of a silenced reporter gene. Here, we present the activity of one of our candidate compounds against the growth of various human lung cancer cell lines and normal bronchial epithelial cells. IC_{50} measurements show the specificity of this drug for cancer vs. normal cells is superior to that of known epigenetic modulators such as trichostatin A and depsipeptide. The compound exhibits excellent potency with IC_{50} values as low as 2 nM. The anticancer potential of this drug is also seen in its ability to inhibit anchorage independent growth in soft agar colony formation assays and to decrease the rate of tumor growth in vivo in mouse xenograft models. Preliminary evidence suggests that in cancer cells this small molecule causes changes in the epigenetic landscape of a subset of genes and alters their expression, without affecting the expression of these genes in normal cells. In addition, this compound is able to modulate nuclear receptor activity in some biological systems, a phenotype which we are currently characterizing.

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ANDROGEN RECEPTOR FUNCTION IS REGULATED BY HISTONE DEMETHYLASES: IMPLICATION FOR PROSTATE CANCER

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Posttranslational modifications of histones such as methylation, acetylation, and phoshorylation regulate chromatin structure and gene expression. Demethylation at distinct lysine residues in histone H3 by lysine-specific demethylase 1 (LSD1) causes either gene repression or activation. As a component of co-repressor complexes, LSD1 contributes to target gene repression by removing mono- and dimethyl marks from lysine 4 of histone H3 (H3K4). In contrast, during androgen receptor (AR)-activated gene expression LSD1 removes mono- and dimethyl marks from lysine 9 of histone H3 (H3K9). Yet, the mechanisms that control this dual specificity of demethylation are unknown. Here, we show that phosphorylation of histone H3 at threonine 6 (H3T6) by protein kinase C beta I (PKCβI) is the key event that prevents LSD1 from demethylating H3K4 during AR-dependent gene activation. *In vitro*, histone H3 peptides methylated at lysine 4 and phosphorylated at threonine 6 are no longer LSD1 substrates. *In vivo*, PKCβI co-localises with AR and LSD1 on target gene promoters and phosphorylates H3T6 upon androgen-induced gene expression. RNAi-mediated knockdown of PKCβI abrogates H3T6 phosphorylation, enhances demethylation at H3K4, and inhibits AR-dependent transcription. Activation of PKCβI requires androgen-dependent recruitment of the gatekeeper kinase protein kinase C (PKC)-related kinase 1 (PRK1). Importantly, elevated levels of PKCβI and phosphorylated H3T6 (H3T6ph) positively correlate with high Gleason scores of prostate carcinomas, and inhibition of PKCβI blocks AR-induced tumour cell proliferation *in vitro* and cancer progression of tumour xenografts *in vivo*. Together, our data establish that androgen-dependent kinase signalling leads to the writing of the novel chromatin mark H3T6ph, which in consequence prevents removal of active methyl marks from H3K4 during AR-stimulated gene expression.

CHIP-SEQ BASED CHARACTERIZATION OF GENES DIFFERENTIALLY RESPONDING TO AR LIGANDS IN ADVANCED PROSTATE CANCER CELLS

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Advanced prostate cancer is treated with anti-androgens, but in most cases, the disease will progress to a more aggressive state called castration resistant prostate cancer (CRPC). CRPC is commonly associated with increased androgen receptor (AR) gene expression. MDV3100, the second-generation anti-androgen drug recently developed by our lab, retains antagonist activity in two CRPC models where the current clinically used first-generation AR antagonist bicalutamide (Casodex) has agonist activity. However, the underlying mechanism is not fully understood.

Here we used an established CRPC cell line LNCaP/AR (LNCaP prostate cancer cells engineered to express higher levels of AR that mimic the clinical scenario) and performed chromatin immunoprecipitation coupled with massively parallel sequencing (ChIP-Seq) to map the AR binding sites in response to R1881 (AR agonist) alone or with a combined treatment with bicalutamide or MDV3100. Out of a total of 42,000 high confidence AR binding sites identified in response to R1881 alone, 32,000 sites are still bound by AR in the presence of bicalutamide, and only 2,000 bound by AR in the presence of MDV3100. Thus, bicalutamide only partially reduces AR DNA binding, while MDV3100 globally blocks AR DNA binding. This result provides a mechanistic explanation of superior antagonism of MDV3100 versus bicalutamide in CRPCs.

By conducting the expression profiling under similar conditions as the ChIP-Seq experiment, we found 309 AR regulated genes (ARGs) with a fold change of two or greater in response to R1881, and most of them contain AR binding sites within 50kb of the TSS. MDV3100 has more profound effect on regulating ARGs compared to bicalutamide.

Overall, this is the first time that the effect of anti-androgens on AR binding has been studied at a genome-wide level.

NOVEL ROLES FOR NUCLEAR RECEPTORS IN BREAST CANCER EPITHELIUM AND STROMA

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Steroid hormone receptors play preeminent roles in the development and progression of breast tumors. In particular, estrogen receptor α (ERα) represents an important prognostic and therapeutic factor in breast cancer. Estrogen-related receptors (ERRα, β and γ) are orphan members of the superfamily of nuclear receptors that share both structural and functional features with the ER, and consequently, may also play a role in the etiology of the disease. In this talk, we will first present a comprehensive view of the functional interaction between ERα and ERRα in breast cancer cells at a genome-wide level with a more precise attention to the events governing the expression of genes contained within the ERBB2 amplicon. Second, we will show that regulation of ERRγ expression in breast cancer cells by a specific miRNA (miR-378) encoded within the PGC-1β gene leads to a reduction in tricarboxylic acid cycle gene expression and oxygen consumption as well as an increase in lactate production and in cell proliferation, indicating that the miR-378/PGC-1β/ERRγ pathway is involved in the orchestration of the Warburg effect in breast cancer cells. Finally, we will demonstrate an unsuspected role for stromal RARβ in promoting mammary gland tumorigenesis suggesting that retinoid-based approaches for the prevention and treatment of solid tumours should be carefully re-evaluated.

SKELETAL MUSCLE METABOLISM: AMPK AND BEYOND.

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During the last decade the AMP-activated protein kinase (AMPK) has emerged as a master regulator of cellular metabolism in many tissues. In skeletal muscle, where much of the work in this area originated, AMPK is potently activated by exercise and AMPK is considered an important regulator of multiple cellular functions. However, emerging data from our laboratory and other groups suggest that AMPK is not essential for many metabolic responses in skeletal muscle. In this talk, studies using knockout and transgenic approaches will be presented that suggest that AMPK may only play a limited role in glucose uptake and fatty acid oxidation in muscle, and some of the adaptations to chronic exercise training. If AMPK is not essential for many of the metabolic responses to exercise, then what are the critical signals? Studies from muscle-specific LKB1 knockout mice provide important clues. In fact, LKB1, the major upstream kinase for AMPK in skeletal muscle, has been shown to be necessary for both the acute effects of muscle contraction on glucose uptake and fatty acid oxidation, as well as some of the adaptations to chronic endurance training. Therefore, other LKB1 substrates, known as AMPK-related kinases, may be central to the effects of exercise on muscle metabolic processes. In particular, studies on sucrose non-fermenting AMPK-related kinase (SNARK), an AMPK-related kinase that has previously been shown to be regulated by multiple cellular stresses in kidney and several tumor cell lines, will be discussed.

GLUCOCORTICOID RECEPTOR AND TRIGLYCERIDE HOMEOSTASIS

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Glucocorticoids play important roles in the regulation of distinct aspects of adipocytes biology. Excess glucocorticoids in adipocytes are associated with various metabolic disorders, including central obesity, insulin resistance and dyslipidemia. Glucocorticoids convey their signals through glucocorticoid receptor (GR), which can occupy genomic glucocorticoid response elements (GREs) to modulate the transcription of its primary target genes. To understand the mechanisms underlying the glucocorticoid effects on adipocytes, we used chromatin immunoprecipitation sequencing to isolate genome-wide GR binding regions (GBRs) in 3T3-L1 adipocytes. We found that many GBRs were located in, or close to genes involved in triglyceride (TG) synthesis, lipolysis, lipid transport and storage. Gene expression analysis and reporter assays confirmed that 14 of these genes are regulated by glucocorticoids and they contain functional GREs. Thus, they are potential GR primary targets (*Scd-1, 2, Gpat3, 4, Agpat2, Lpin1, Lipe, Mgll, Angptl4, Cd36, Lrp-1, Vldlr, Slc27a2, S3-12*). Although TG levels were not significantly increased by glucocorticoid treatment, stable isotope labeling technique revealed that 4-day glucocorticoid treatment induced the rate of TG synthesis and lipolysis, at the same time, in inguinal fat. This observation was in agreement with our finding that glucocorticoids activate genes involved in both TG biosynthetic and lipolytic pathways. Notably, we found that 9 of these 12 genes were induced in transgenic mice over-expressing corticotropin-releasing hormone, which have elevated plasma glucocorticoid levels. These results suggested that a similar mechanism was used to regulate TG homeostasis during chronic glucocorticoid treatment. To further test whether these potential GR primary target genes indeed mediate glucocorticoid-regulated lipid homeostasis in vivo, knockout mice lacking specific potential GR primary target gene were used. Mice lacking Angptl4 gene have impaired glucocorticoid-induced lipolysis in inguinal fat. This is likely due to an impaired glucocorticoid-induced PKA signaling. Moreover, these mice are protected from glucocorticoid-induced hyperlipidemia and fatty liver. In summary, our studies identified a glucocorticoid-controlled gene network involving in the regulation of TG homeostasis in adipocytes. The dissection of the mechanisms governing this network should provide important insights into the therapeutic approaches against metabolic diseases.

A HUMAN PPARγ MUTATION CAUSES INHERITED DIABETES THROUGH A UNIQUE MECHANISM OF ACTION.

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Peroxisome proliferator-activated receptor gamma (PPARγ) is a class II nuclear receptor that plays a central role in adipose tissue differentiation and regulates lipid metabolism and insulin sensitivity in multiple tissues. PPARγ activation by thiazolidinediones is widely employed in clinical practice to improve insulin sensitivity. Human loss-of-function mutations in PPARγ cause familial partial lipodystrophy (FPLD), a rare disorder characterized by redistribution of adipose tissue and severe early-onset diabetes. Detailed study of several such PPARγ mutations has improved our understanding of the role of PPARγ in the pathophysiology of diabetes. A recently identified PPARγ mutant, E157D, causes FPLD in a large Canadian family. Glutamate 157, located in the P-box region of the DNA binding domain of PPARγ, interacts with the major groove of the DNA molecule and has been shown to be important for PPAR response element (PPRE) selectivity. Although the Glu>Asp mutation would not be predicted to disrupt the local conformation of the protein, it clearly causes a severe phenotype with dominant inheritance. We hypothesized that E157D PPARγ has altered binding site selectivity and regulates a different set of target genes than the wild-type receptor. We assessed E157D PPARγ activity on a small set of well-characterized PPREs. When ectopically expressed in NIH3T3 fibroblasts, the mutant receptor was generally defective at driving gene expression from heterologous transcription reporters as well as endogenous PPREs. The severity of this transcriptional defect was not uniform across all PPREs, ranging from full induction to two hundred-fold lower activity compared to the wild-type receptor. Surprisingly, the E157D mutation did not affect PPARγ binding affinity for PPREs in an electrophoretic mobility shift assay. Contrary to our original hypothesis, this PPARγ mutant binds DNA as well as the wild-type receptor, but fails to fully activate gene transcription. We conclude that the E157D mutation disrupts a post-DNA binding regulatory step in the transcription activation cascade and that the degree of this disruption is affected by the DNA sequence to which the receptor is bound. Our findings suggest a previously unrecognized, complex and finely tuned interaction between the nuclear receptor DNA binding domain and other members of the transcriptional machinery.

COACTIVATORS AND DISEASE: ABLATION OF SRC-3 PHENOCOPIES A MYOPATHY RESEMBLING HUMAN CACT DEFICIENCY

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Accumulating evidence has established a clear role for the Steroid Receptor Coactivators (SRCs) in the regulation of systems metabolism. To gain a more global understanding of how the SRCs dynamically influence the metabolic landscape, we performed comprehensive metabolomics analysis upon ablation of each coactivator. These data revealed a multitude of tissue- and energy state-dependent functions for each of the three coactivators. Of particular interest, loss of SRC-3 leads to a marked increase in long and very-long chain acyl-carnitines, specifically in muscle. We have correlated the loss of SRC-3 with the muscle-specific loss of expression of the essential carnitine/acyl-carnitine translocase (CACT). Phenotypically, SRC-$3^{-/-}$ mice resemble humans with a deficiency in this transporter, highlighting the physiological importance of SRC-3 in muscle. Ablation of SRC-3 in mice phenocopies the major hallmarks of human CACT deficiency including: elevated long/very-long chain acyl-carnitines, hypoglycemia, muscle weakness, hypoketonemia, cardiac arrhythmia, and hyperammonemia. Collectively, these studies provide the first evidence for a muscle-specific function of SRC-3 and perhaps identify SRC-3 as a new therapeutic target for the treatment of muscle-related and metabolic disorders.

EPIGENOMIC REGULATION OF CIRCADIAN RHYTHM AND METABOLISM BY NUCLEAR RECEPTORS

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Nuclear receptors transduce environmental and metabolic signals into alterations in gene expression by recruiting coregulators that alter chromatin structure. Rev-erbα (NR1D1) is expressed in a circadian manner and constitutes a negative limb of the circadian clock, primarily by repressing the expression of Bmal1. The repressive function of Rev-erbα is mediated by a corepressor complex that is anchored by Nuclear Receptor Corepressor (NCoR) and contains the epigenetic modification enzyme, histone deacetylase 3 (HDAC3). The enzyme activity of HDAC3 requires binding to NCoR via a SANT-motif-containing Deacetylase Activating Domain (DAD). Mice harboring an NCoR DAD mutation that prevents HDAC3 binding have dysregulated circadian rhythms and alterations in metabolism that demonstrate the importance of epigenomic regulation, and in particular the NCoR-HDAC3 interaction, in normal metabolic and circadian physiology. The activity of Rev-erbα is controlled by binding of heme ligand, by lithium-modulated degradation, and by a dramatic circadian expression pattern. Genome-wide analysis of Rev-erbα and HDAC3 binding in metabolic tissues reveals cistromes that include a large number of metabolic genes, and strongly suggest a key role of the Rev-erbα-NCoR-HDAC3 axis in the normal circadian physiology of metabolism. The nuclear receptor PPARγ is the target of antidiabetic thiazolidinedione (TZD) drugs, and a transcriptional regulator of lipid metabolism. PPARγ is also required for adipogenesis. On a genome-wide scale, identification of PPARγ cistromes in adipocytes as well as macrophages has revealed tissue-specific transcription factors and epigenomic marks that selectively target PPARγ to pathway-related DNA binding sites. The epigenomic regulation and functions of Rev-erbα and PPARγ highlight the key role of nuclear receptors in the integrated physiology of circadian rhythm and metabolism.

HOMEOSTATIC LEVELS OF SRC-2 AND SRC-3 PROMOTE EARLY HUMAN ADIPOGENESIS

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The related coactivators SRC-2 and SRC-3 interact with PPARγ to coordinate and amplify a transcriptional circuit that promotes adipogenesis. To identify potential coactivator interplay and redundancy during human adipogenesis, we utilized high content analysis at the single cell level to quantify new links between PPARγ, SRC-2, SRC-3 and lipogenesis in human subcutaneous adipocytes. As expected during the first 96 h of differentiation, there were robust and concomitant increases in PPARγ protein levels and lipid content within the bulk population. When examined on a cell-to-cell basis, marked cell-to-cell heterogeneity exists, with PPARγ and lipid levels varying up to a 1000-fold, which were further subdivided into $PPAR\gamma^{hi}/Lipid^{hi}$, $PPAR\gamma^{hi}/Lipid^{lo}$, $PPAR\gamma^{lo}/Lipid^{hi}$, $PPAR\gamma^{lo}/Lipid^{lo}$ states to understand population kinetics during differentiation. In contrast, protein levels of SRC-2 and SRC-3 exhibited significantly less cell-to-cell variability. By a complementary FRET imaging approach, SRC-2 and SRC-3 interact with PPARγ without significant increases upon treatment with differentiation cocktail. Collectively, we hypothesized that permissive levels of SRC-2 and SRC-3 were necessary for adipogenesis. To probe these coactivator thresholds, we down-regulated SRC-2 and SRC-3 while, simultaneously, quantifying PPARγ. Knockdowns of SRC-2 and SRC-3, individually or jointly, equally inhibited lipid accumulation by preventing lipogenic gene engagement, without affecting PPARγ protein levels. Supporting a dominant pro-adipogenic role for SRC-2 and SRC-3, knockdown of the other homologous p160, SRC-1, did not affect human adipogenesis. SRC-2 and SRC-3 knockdown increased the proportion of cells in a $PPAR\gamma^{hi}/Lipid^{lo}$ state while synergistically increasing phospho-PPARγ−S114, found to reduce PPARγ transcriptional activity and block adipogenesis. Together, these studies demonstrate that SRC-2 and SRC-3 concomitantly promote human adipocyte differentiation, by attenuating phospho-PPARγ−S114, and modulating PPARγ cellular heterogeneity.

METABOLOMICS APPLIED TO UNDERSTANDING OF NUCLEAR RECEPTOR BIOLOGY AND METABOLIC DISEASE

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We seek to apply comprehensive metabolic analysis tools (sometimes called "metabolomics") for understanding of mechanisms underlying chronic human diseases such as diabetes, obesity, and cardiovascular disease. Current approaches include metabolic profiling of important groups of metabolic intermediates by both "targeted" and "unbiased" mass spectrometry (in collaboration with Drs. James Bain, Robert Stevens, Olga Ilkayeva, Brett Wenner, Michael Muehlbauer, Mark Butler, and David Millington at Duke). These tools have been used to investigate the metabolic mechanisms underlying development of peripheral insulin resistance in animals and humans. For example, we have recently identified perturbations of branched chain amino acid (BCAA) catabolism in multiple cohorts of insulin resistant humans compared to normally insulin sensitive controls and have translated these findings to rodent models to demonstrate a contribution of BCAA to development of insulin resistance that is independent of body weight. We have also collaborated with Dr. Bert O'Malley and colleagues to investigate the metabolic impact of the transcriptional co-regulators SRC-1, SRC-2, and SRC-3. In this study, metabolic profiling allowed us to pinpoint the tissues and specific metabolic pathways impacted upon knock-out of each of the SRC family members. Finally, in collaboration with Dr. Alan Attie at the University of Wisconsin, we have integrated transcriptomic and metabolomic analysis to identify new pathways that control hepatic gluconeogenesis and PEPCK expression. These examples will serve to illustrate the potential of comprehensive metabolic profiling methods for providing insights into nuclear receptor biology and metabolic disease mechanisms.

NUCLEAR RECEPTOR REPRESSION PATHWAYS THAT REGULATE MICROGLIA AND ASTROCYTE ACTIVATION

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Emerging evidence suggests that sustained inflammatory responses involving microglia and astrocytes contribute to the pathogenesis of neurodegenerative diseases that include Alhzheimer’s disease, Parkinson’s disease and Multiple Sclerosis. Microglia are the major resident immune cells in the brain, where they constantly survey the microenvironment and produce factors that influence surrounding glia and neurons. Astrocytes play key roles in neuronal support and tissue homeostasis within the CNS and also respond to infection and injury, with recent studies suggesting that communication between microglia and astrocytes can function to greatly amplify inflammation. Microglia and astrocytes switch from deactivated to activated phenotypes upon pathogen invasion or tissue damage and thereby promote an inflammatory response that serves to engage the immune system and initiate tissue repair processes. In most cases, these responses are self-limited, resolving once infection has been eradicated and tissue damage repaired. However, persistence of an inflammatory stimulus and/or failure in normal resolution mechanisms results in chronic inflammation that can contribute to neurotoxicity. Recent studies suggest that nuclear receptors and their ligands regulate pathogenic mechanisms that underlie neurodegeneration, including neurotoxic inflammation. Our studies have recently focused on defining signal-dependent transcriptional repression pathways that are utilized by Nurr1 and estrogen receptor beta to negatively regulate inflammatory gene expression in microglia and astrocytes. We propose that these pathways contribute to maintenance of normal tissue homeostasis within the central nervous system and become dysregulated in the context of neurodegenerative disease.

PROTECTIVE ROLE OF ENDOTHELIAL PPARΓ IN ATHEROSCLEROSIS

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The nuclear receptor PPARγ has been shown to regulate glucose and lipid metabolism, and inflammation. Activation of PPARγ by ligand treatment leads to an improvement of atherosclerosis in multiple animal and human studies. This is mediated partially by macrophage PPARγ as it increases cholesterol efflux from the macrophage. However, as endothelium is involved in the development of atherosclerosis, we hypothesized that endothelial PPARγ plays a role in atherogenesis as well. To study this, we first replace the bone marrow of Tie2-cre driven PPARγ f/f mice in the LDL receptor null background and their littermate controls with PPARγ wildtype GFP+ bone marrow. This generates an endothelial specific knockout of PPARγ. After feeding them atherogenic diet for various periods of time, these mice were harvested for atherosclerotic lesion analyses. While endothelial PPARγ knockout mice and littermates have comparable blood pressure, fasting glucose, and weights, lesion analyses showed endothelial PPARγ knockout mice have 51% and 29% more lesion at the aortic sinus after 8 and 16 weeks of atherogenic diet, respectively ($p<0.05$). These results demonstrate that endothelial PPARγ plays an athero-protective role. To examine whether endothelial PPARγ is involved in early stages of atherosclerotic lesion development, we scored GFP+ macrophage infiltration into the lesser curvature of the aorta and found endothelial PPARγ knockout mice have 75% more macrophage infiltration and increased serum levels of soluble ICAM-1 and E-selectin after 2 weeks of atherogenic diet. To understand the function of endothelial PPARγ, we performed microarray analyses using primary endothelial cells isolated from mice before and two weeks after the initiation of atherogenic diet and found that the endothelial cells lacking PPARγ on chow diet exhibit a gene expression profile similar to wildtype endothelial cells after two weeks of atherogenic diet, suggesting that PPARγ knockout endothelial cells are already activated. Among the genes upregulated is the cytokine fractalkine, which is involved in both chemo-attraction and adhesion of leukocytes to the endothelium. *In vitro* studies confirm the increased ability for the PPARγ knockout endothelial cells to adhere to leukocytes comparing to wildtype cells and while this is not suppressed by rosiglitazone, it can be blocked by fractalkine neutralizing antibodies. In conclusion, deficiency of endothelial PPARγ leads to endothelial activation, increasing expression of adhesion molecules and cytokines, and consequently more severe atherosclerosis.

INFLAMMATION, ENDOPLASMIC RETICULUM STRESS, AND METABOLIC CONTROL

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Chronic, low grade, inflammatory responses in metabolically active sites such as adipose tissue is a hallmark of chronic metabolic disease, especially obesity. This heightened metabolically triggered chronic inflammation or "metaflammation/paraflammation" is a critical link between obesity and other associated pathologies, such as insulin resistance, type 2 diabetes, hepatosteosis, cardiovascular disease and other pathologies. In metabolic context, inflammatory cascades involve both metabolic cells such as adipocytes and hepatocytes as well as immune effectors such as macrophages, mast cells and T cells. The collective activity of the inflammatory networks intersects with insulin receptor signaling at several levels to block insulin action and disrupt glucose and lipid metabolism. Our earlier work has identified the activation of inflammatory kinases such as c-Jun N-terminal kinase (JNK) as a critical event seen in all forms of insulin resistance. Our studies, in search for intrinsic pathways leading to JNK activation and metabolic dysfunction, led to the discovery of endoplasmic reticulum (ER) dysfunction as a critical mechanism underlying metabolic disease, especially obesity and diabetes. Recently, we identified protein kinase R (PKR) as a potential intersection point between nutrients, organelle dysfunction and metabolic control. Using these systems, we are now pursuing the precise mechanisms by which nutrient sensing and metabolism intersect with immune response and organelle stress and identify the molecular targets of specific nutrients in their ability to engage components of the innate immune response. Here, I will present the latest developments and translational opportunities emerging from these platforms that are applicable to human metabolic diseases.

A DUAL ACTION OF GLUCOCORTICOIDS ON THE TYPE I INTERFERON NETWORK

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Dysregulated cytokine signaling is a key contributor to autoimmunity and inflammation; in particular, Type I Interferon (IFNα/β) has been causally linked to systemic lupus. Most autoimmune diseases are principally managed with glucocorticoids (GCs) which block proinflammatory cytokine gene expression. With respect to IFN, we have previously shown that activated glucocorticoid receptor (GR) controls the activity of interferon regulatory factor (IRF)3, a critical regulator of IFNα4/β gene transcription (Reily et al, *EMBO J* 2006). Interestingly, unlike their *production*, cytokine *signaling* through their cognate Jak/STAT pathways is reportedly unaffected or even stimulated by GCs. Unexpectedly, we found that dexamethasone (Dex) specifically attenuates the IFN-induced gene expression in primary macrophages. To identify the Dex-sensitive step in the IFN-Jak/STAT signaling pathway, we evaluated the function of the heterotrimeric STAT1:STAT2:IRF9 (ISGF3) transcription complex, responsible for mediating IFN signals. Interestingly, we found that ISGF3 utilized the GR cofactor GRIP1/TIF2 as a coactivator. Indeed, dominant negative GRIP1, its siRNA-mediated knockdown, genetic disruption or depletion by GC-activated GR attenuated ISGF3 promoter occupancy, preinitiation complex assembly and ISG transcription, whereas GR antagonist RU486, which disrupts the GR:GRIP1 interaction, restored IFN response. Thus, type I IFN represents a unique cytokine controlled by GR at the levels of both production (*e.g.*, IFN gene transcription) and signaling, through the antagonism with ISGF3 effector function, revealing a novel facet of the immunosuppressive properties of GCs.

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DELETION OF NUCLEAR RECEPTOR COREPRESSOR PROTEIN (NCOR) IN ADIPOSE TISSUE IMPROVES SYSTEMIC INSULIN RESISTANCE IN DIET-INDUCED OBESE MICE

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Nuclear receptor corepressor protein (NCoR) serves as a corepressor for nuclear receptors and other factors and recent evidence suggests that NCOR1 can be a regulator of metabolism. NCoR represses PPARγ (peroxisome proliferator-activator receptor gamma) mediated transcriptional activity in 3T3-L1 adipocytes in vitro, but its role in vivo remains unclear. We generated adipocyte specific NCoR1 knock-out (NCoR-ap2) mice by crossing NCoR f/f with ap2-Cre mice, to investigate the function of NCoR in insulin sensitivity. Our data show that NCoR-ap2 mice gain more weight on a 60% high fat diet (HFD) with increased accumulation of subcutaneous and visceral fat. The KO mice also display reduced plasma free fatty acid (FFA) and insulin levels. Insulin tolerance and glucose tolerance tests showed that NCoR-ap2 mice are more glucose tolerant compared with wt HFD mice. Hyperinsulinemic-euglycemic clamp studies demonstrate that the NCoR-ap2 mice have higher insulin stimulated glucose disposal rate (IS-GDR), suppression of hepatic glucose production (HGP) and FFA suppression, showing that NCoR deletion in adipocytes leads to improved systemic insulin sensitivity in all three major insulin target tissues (liver, muscle and fat). Hepatic triglyceroride content and steatosis were reduced in the KO mice, while in adipose tissue, there was less macrophage infiltration, reduced inflammatory gene expression (including, IL-1β, iNOS and IL-12p40), but greater adipogenic gene expression (including, SCD1, FAS, ACC and SREBP-1c) in the KOs. The effects of rosiglitazone treatment to improve in vivo insulin sensitivity were blunted in the NCoR-ap2 mice, indicating that NCoR-ap2 mice are relatively refractory to PPARγ stimulation. Together, these results demonstrate that NCoR-dependent transrepression is a key determinant of the adipogenic set point as well as an integrator of glucose metabolism and whole-body metabolic homeostasis.

IDENTIFICATION OF STAT6 AS A REQUIRED LICENSING FACTOR FOR PPARγ-REGULATED GENE EXPRESSION IN MACROPHAGES AND DENDRITIC CELLS

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Cell types and subtypes are specified upon receiving external stimuli and also by endogenous programmes. The mechanisms of differentiation and subtype specification of certain immune cells such as macrophages are critical to understand the regulatory logic and principles of the immune system as a whole. It is becoming more and more appreciated that besides cytokines lipid signaling also contributes to these processes. Peroxisome proliferator-activated receptor γ (PPARγ), a lipid-activated transcription factor regulates the interplay of lipid metabolism and inflammation in macrophages and dendritic cells (DCs). These immune cells if exposed to distinct inflammatory milieu show cell type specification. We will present evidence to show one of the the mechanisms how PPARγ signaling is regulated in these distinct subsets of cells. We show that proinflammatory molecules inhibit, while interleukin-4 (IL-4) stimulates PPARγ activity in macrophages and DCs. Furthermore, IL-4 signaling augments PPARγ activity through a novel interaction between PPARγ and Signal Transducer and Activators of Transcription 6 (STAT6) on promoters of PPARγ target genes, including FABP4/aP2. STAT6 acts as a required licensing factor for PPARγ by promoting DNA binding and consequently increasing the number of regulated genes and also the magnitude of responses. This mechanism, underpinning cell type-specific responses, represents a unique way of controlling nuclear receptor signaling by inflammatory molecules.

DYNAMIC EXCHANGE PREVENTS RECEPTOR COMPETITION ON REGULATORY ELEMENTS

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The glucocorticoid receptor (GR), like many DNA-binding transcription factors, dynamically interacts with response elements (REs) to regulate temporal gene expression patterns that are required for development and homeostasis. Live cell microscopy studies demonstrate that GR resides on glucocorticoid REs (GREs) for time scales of seconds or less, indicating a “hit and run” mechanism in which factors stochastically interact with binding sites, modify the chromatin, and then rapidly leave the site. In contrast, many current interpretations of steady-state chromatin immunoprecipitation (ChIP) results suggest that steroid receptors bind on time scales of minutes to hours. These longer residency times are proposed by others to promote sequential, ordered formation of multi-protein regulatory complexes, which are predicted to saturate response elements (Degenhardt et al., 2009). Using both microscopy and ChIP approaches, we show that the concurrent binding of a second GRE-targeting receptor, the estrogen receptor pBox mutant (ER pBox), does not reduce the GRE binding of GR or ER pBox compared to the individual receptor steady-state binding levels. In mathematical binding simulations, this lack of competition is explained by short GR/GRE residency times and relatively long times between binding events, which produce GREs that are far from saturation. At many genomic sites where GR-binding causes chromatin to become more accessible to nuclear proteins, the steady-state levels of concurrently binding ER pBox are also increased. This suggests that accessible GR-dependent chromatin remodeling states persist after GR has left the GRE, providing a mechanism for GR to stimulate transcription between intermittent binding events. The prevalence of sparsely occupied GREs during transcriptional activation is a novel biophysical condition which should be included in future quantitative models of dynamic gene expression. These findings further support the “hit and run” transcription hypothesis and argue against stable chromatin binding by actively engaged regulatory complexes.

LESSONS FROM PPARγ NULL MICE, FROM DEVELOPMENT TO METABOLISM

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The effects of global deletion of Peroxisome Proliferator Activated Receptor gamma (PPARγ) on development could not be explored so far because of the lethality of the germ-line deletion of PPARγ. The presence of a major alteration of the placental development seemed the main cause of this early embryonic lethality. Therefore, we used an epiblast-specific deletion of the PPARγ alleles to preserve PPARγ expression in the trophoblast. This allowed us to obtain fully viable PPARγ null mice, thereby demonstrating that the placental defect is the unique cause of PPARγ-/- early embryonic lethality.

To analyse the activity of PPARγ in the later stage of placental development when its expression peaks, we treated pregnant wild-type mice with the PPARγ agonist rosiglitazone. This treatment resulted in an aberrant placental microvasculature and disorganisation of the placental layers accompanied by the decreased expression of proangiogenic genes such as PLF, vascular endothelial growth factor and Pecam1. These findings demonstrate that PPARγ plays a pivotal role in controlling vascular proliferation and contributes to its quiescence in late pregnancy.

As mentioned above, we are now able to obtain a global knockout mouse model of PPARγ. The PPARγ null mice are born alive, along the expected mendelian ratio. They are totally lacking adipose tissue and are particularly fragile before weaning after which their growth not only catch back but exceed that of their control littermates. The exploration and main observations of this new mouse model will be presented.

GENOMIC ANALYSES OF ESTROGEN-REGULATED TRANSCRIPTION REVEAL NEW FACETS OF THE ESTROGEN SIGNALING PATHWAY

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Estrogens play crucial roles in regulating the expression of hundreds, perhaps thousands, of genes in normal physiological states, such as the development of reproductive organs, as well as disease states, such as breast cancers. Although recent genomic analyses of estrogen-dependent transcription have provided the vast amount of information, many aspects of the estrogen-dependent gene regulatory network, however, have not yet been elucidated. Foremost among these is the set of genes comprising the set of direct or primary estrogen-regulated targets. This information is critical because the direct estrogen-regulated transcriptome is likely to define a useful set of biomarkers with prognostic value and utility as therapeutic targets in breast cancers.

To define the direct estrogen-regulated transcriptome with greater sensitivity than existing genomic analyses, we have applied an innovative new method called Global Nuclear Run-On and Massively Parallel Sequencing (GRO-seq) to explore the estrogen-regulated transcriptome in MCF-7 breast cancer cells. GRO-seq is a deep sequencing-based genomic approach that provides a genome-wide map of the location and orientation of transcriptionally engaged RNA polymerase II (Pol II). Using GRO-seq as a direct measure of transcriptional output, we have produced libraries from MCF-7 breast cancer cells in response to short treatments with 17-estradiol (E2) that reveal the location of actively transcribing Pol II in human breast cancer cells. Our goals are to (1) identify in an unbiased manner the annotated and unannotated transcripts that change expression during estrogen signaling, (2) obtain a time-course of activation for each transcript, and (3) identify the direct estrogen target genes. Our results have allowed us to detect E2-regulated transcripts with greater sensitivity and at much earlier time points than has been achieved with conventional expression microarray platforms. Collectively, these studies are revealing many unexpected novel features of E2-regulated gene expression, as well as new mechanistic insights. We expect that genome-wide inferences based on the direct estrogen-regulated transcriptome will be useful for understanding the molecular mechanisms driving the development and progression of breast cancers.

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THE P23 MOLECULAR CHAPERONE AND GCN5 ACETYLTRANSFERASE COOPERATIVELY MODULATE THE STABILITY OF PROTEIN-DNA COMPLEXES

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Steroid hormone receptors are reliant on the Hsp90 molecular chaperone system to establish and maintain both a hormone-binding competent state and a dynamic interaction with promoter response elements. By modulating the stability of receptor-DNA complexes, molecular chaperones facilitate the signaling process by ensuring efficient transitions between the employed coactivator complexes and permitting effective sensing of the hormonal signal. The Hsp90 cochaperone p23 appears to drive the disassembly of receptor-bound coactivator complexes. While dynamic action is likely critical for proper gene regulation, the mechanisms used to achieve the fast dissociation-kinetics are not fully understood.
To initially investigate a p23 cochaperone we focused on the yeast homolog Sba1 and exploited high-throughput genomic and proteomic approaches to gain a global functional map of a p23 protein. Our studies revealed a broad nuclear role for Sba1, contrasting with the historical dogma of restricted cytosolic activities for chaperones. Importantly, we found that the roles were conserved since mammalian p23 also affected the identified pathways. To understand how a mammalian p23 cochaperone intersects with a nuclear protein we followed up on the synthetic lethal genetic interaction between sba1Δ and gcn5Δ; GCN5 encodes for a histone acetyltransferase protein. Surprisingly, the connection does not involve chromatin events. Rather, we found that p23 and Gcn5 form a regulatory circuit that coordinately regulates the DNA-bound state of diverse proteins. Using both the Glucocorticoid Receptor (GR) and Heat Shock Factor 1 (HSF1) as model targets, we observed that both p23 and Gcn5 inhibited GR and HSF1 DNA binding activity. p23 initiated disassembly of DNA-bound transcription factors while Gcn5 prolonged the dissociated state by acetylating a lysine residue within the DNA binding domain. In addition, p23 can control the enzymatic activity of Gcn5, which likely serves to close the regulatory circuit. Notably, p23 and Gcn5 affected the DNA binding activities of a variety of proteins including other transcription factors and proteins required for DNA replication and telomere maintenance. Our data support a model in which p23 and Gcn5 constitute a general system for modulating protein DNA dynamics across the genome.

TLE3 IS A DUAL FUNCTION TRANSCRIPTIONAL COREGULATOR OF ADIPOGENESIS

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PPARγ and Wnt signaling are central positive and negative regulators of adipogenesis, respectively. Here we identify the groucho family member TLE3 as a transcriptional integrator of the PPARγ and Wnt pathways. TLE3 is a direct target of PPARγ that participates in a positive feedback loop during adipocyte differentiation. TLE3 coactivates PPARγ and acts synergistically with PPARγ on its target promoters to stimulate adipogenesis. At the same time, induction of TLE3 during differentiation provides a mechanism for termination of Wnt signaling. TLE3 antagonizes TCF4 activation by β-catenin in preadipocytes, thereby inhibiting Wnt target gene expression and reversing β-catenin-dependent repression of adipocyte gene promoters. Transgenic expression of TLE3 in adipose tissue *in vivo* mimics the effects of PPARγ agonist and ameliorates high fat diet-induced insulin resistance. Our data suggest that TLE3 acts as a dual function switch, driving the formation of both active and repressive transcriptional complexes that facilitate the adipogenic program.

SUMOYLATION OF *C. ELEGANS* NUCLEAR RECEPTOR NHR-25 PROMOTES PROPER ORGANOGENESIS

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We are using *C. elegans* to deconvolute how NRs govern cell- and tissue-specific gene regulatory programs. In particular, we focus on the single *C. elegans* NR5A family receptor, NHR-25, and its coordination of diverse events such as molting, seam cell differentiation, dauer formation, and somatic gonad and vulva formation. To uncover novel co-regulators of NHR-25 activity we performed a genome-wide yeast two-hybrid (Y2H) screen. NHR-25 interacts strongly with SMO-1, the single *C. elegans* SUMO-1 homolog (SMO-1). To distinguish between covalent sumoylation and non-covalent interaction we performed mutational analysis. Interestingly, deletion of the SMO-1 C-terminal di-glycine repeat and mutations in the SMO-1 beta sheet, which abrogate covalent sumoylation and non-covalent binding, respectively, prevented NHR-25 binding in Y2H assays. One possible model is that NHR-25 initially binds SMO-1 non-covalently to promote sumoylation of NHR-25, in a mechanism similar to thymine DNA glycosylase sumoylation. *smo-1* deletion mutants undergo aberrant vulval morphogenesis resulting in a single protrusion at the site of the vulva (Pvl). Additionally, *smo-1* mutants have a weak multivulva (Muv) phenotype where a single proper vulva develops and is surrounded by ventral protrusions comprised of vulval tissue. We observed a genetic interaction between *nhr-25* and *smo-1*: *nhr-25(RNAi)* in *smo-1(ok359)* mutant worms exacerbates the *smo-1* multivulva phenotype. Interestingly, *nhr-25(RNAi)* prevents the vulval precursor cells from completing full rounds of cell division, suppressing the Pvl phenotype. These data suggest a critical role for receptor modification by sumoylation in execution of an organogenesis program. Given the impact of sumoylation on target gene recognition by the vertebrate homologs of NHR-25, SF-1 and LRH-1, these studies provide insight into the role of sumoylation on NR5A receptor-governed physiological programs.

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DYNAMICS OF NUCLEAR RECEPTOR INTERACTIONS AT GLOBAL REGULATORY ELEMENTS

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Multiple cyclical mechanisms are involved in the transcriptional regulation of gene expression by nuclear receptors. Live cell imaging analysis demonstrates that receptors exchange rapidly with regulatory elements in the continued presence of ligand [Mol Cell 35:741 (2009)]. In some cases, ligand concentrations in the animal are also subject to oscillatory patterns. We have also shown that transcription factors can be actively displaced from the nucleoprotein template during chromatin remodeling in vitro [Mol Cell 14:163 (2004)]. To examine the complex dynamic of factor/template interactions on the global scale, we have carried out a genome-wide analysis of nuclear receptor binding events, as well as a global characterization of DNaseI hypersensitive transitions (DHS) associated with receptor binding [Mol Cell 29:611 (2008)]. We find that receptor/genome interactions are universally associated with local chromatin remodeling events. The presence of these locally open chromatin regions is highly cell specific, and receptor binding correlates well with the presence of these sites. We have examined the ability of factors that recognize the same response element to compete for binding, by direct imaging of site specific binding events in living cells, and by ChIP analysis at genome wide DHS sites. In contrast to competition, we observe assisted loading of factors. These findings will be discussed in terms of 1) the "hit-and-run" model first proposed for the glucocorticoid receptor, 2) the central role for chromatin remodeling in genome-wide action by nuclear receptors, and 3) the function of dynamic processes operating on multiple time scales in the evolution of complex transcriptional programs.

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EMERGENCY	CSHL	BANBURY
Fire	**(9) 742-3300**	**(9) 692-4747**
Ambulance	**(9) 742-3300**	**(9) 692-4747**
Poison	**(9) 542-2323**	**(9) 542-2323**
Police	**(9) 911**	**(9) 549-8800**
Safety-Security	**Extension 8870**	

Emergency Room **Huntington Hospital** 270 Park Avenue, Huntington	**631-351-2300** **(1037)**
Dentists Dr. William Berg Dr. Robert Zeman	 **631-271-2310** **631-271-8090**
Doctor MediCenter 234 W. Jericho Tpke., Huntington Station	**631-423-5400** (**1034**)
Drugs - 24 hours, 7 days Rite-Aid 391 W. Main Street, Huntington	**631-549-9400** (**1039**)

Free Speed Dial
Dial the four numbers (****) from any **tan house phone** to place a free call.

GENERAL INFORMATION

Books, Gifts, Snacks, Clothing, Newspapers
BOOKSTORE 367-8837 (hours posted on door)
Located in Grace Auditorium, lower level.

Photocopiers, Journals, Periodicals, Books, Newspapers
Photocopying – Main Library
Hours: 8:00 a.m. – 9:00 p.m. Mon-Fri
10:00 a.m. – 6:00 p.m. Saturday
Helpful tips - Obtain PIN from Meetings & Courses Office to enter Library after hours. See Library staff for photocopier code.

Computers, E-mail, Internet access
Grace Auditorium
Upper level: E-mail only
Lower level: Word processing and printing.
STMP server address: mail.optonline.net
To access your E-mail, you must know the name of your home server.

Dining, Bar
Blackford Hall
Breakfast 7:30–9:00, Lunch 11:30–1:30, Dinner 5:30–7:00
Bar 5:00 p.m. until late
Helpful tip - If there is a line at the upper dining area, try the lower dining room

Messages, Mail, Faxes
Message Board, Grace, lower level

Swimming, Tennis, Jogging, Hiking
June–Sept. Lifeguard on duty at the beach. 12:00 noon–6:00 p.m.
Two tennis courts open daily.

Russell Fitness Center
Dolan Hall, west wing, lower level
PIN#: **Press 64485 (then enter #)**

Concierge
On duty daily at Meetings & Courses Office.
After hours – From tan house phones, dial x8870 for assistance

Pay Phones, House Phones
Grace, lower level; Cabin Complex; Blackford Hall; Dolan Hall, foyer

CSHL's Green Campus

Cold Spring Harbor Laboratory is pledged to operate in an environmentally responsible fashion wherever possible. In the past, we have removed underground oil tanks, remediated asbestos in historic buildings, and taken substantial measures to ensure the pristine quality of the waters of the harbor. Water used for irrigation comes from natural springs and wells on the property itself. Lawns, trees, and planting beds are managed organically whenever possible. And trees are planted to replace those felled for construction projects.

Two areas in which the Laboratory has focused recent efforts have been those of waste management and energy conservation. The Laboratory currently recycles most waste. Scrap metal, electronics, construction debris, batteries, fluorescent light bulbs, toner cartridges, and waste oil are all recycled. For general waste, the Laboratory uses a "single stream waste management" system, removing recyclable materials and sending the remaining combustible trash to a cogeneration plant where it is burned to provide electricity, an approach considered among the most energy efficient, while providing a high yield of recyclable materials.

Equal attention has been paid to energy conservation. Most lighting fixtures have been replaced with high efficiency fluorescent fixtures, and thousands of incandescent bulbs throughout campus have been replaced with compact fluorescents. The Laboratory has also embarked on a project that will replace all building management systems on campus, reducing heating and cooling costs by as much as twenty-five per cent.

Cold Spring Harbor Laboratory continues to explore new ways in which we can reduce our environmental footprint, including encouraging our visitors and employees to use reusable containers, conserve energy, and suggest areas in which the Laboratory's efforts can be improved. This book, for example, is printed on recycled paper.

1-800 Access Numbers

AT&T	**9-1-800-321-0288**
MCI	**9-1-800-674-7000**

Local Interest

Fish Hatchery	631-692-6768
Sagamore Hill	516-922-4447
Whaling Museum	631-367-3418
Heckscher Museum	631-351-3250
CSHL DNA Learning Center	x 5170

New York City

Helpful tip -

Take Syosset Taxi to Syosset Train Station ($8.00 per person, 15 minute ride), then catch Long Island Railroad to Penn Station (33rd Street & 7th Avenue). Train ride about one hour.

TRANSPORTATION

Limo, Taxi

Syosset Limousine	516-364-9681 (**1031**)
Super Shuttle	800-957-4533 (**1033**)
To head west of CSHL - Syosset train station	
Syosset Taxi	516-921-2141 (**1030**)
To head east of CSHL - Huntington Village	
Orange & White Taxi	631-271-3600 (**1032**)
Executive Limo	631-696-8000 (**1047**)

Trains

Long Island Rail Road	822-LIRR
Schedules available from the Meetings & Courses Office.	
Amtrak	800-872-7245
MetroNorth	800-638-7646
New Jersey Transit	201-762-5100

Ferries

Bridgeport / Port Jefferson	631-473-0286 **(1036)**
Orient Point/ New London	631-323-2525 **(1038)**

Car Rentals

Avis	631-271-9300
Enterprise	631-424-8300
Hertz	631-427-6106

Airlines

American	800-433-7300
America West	800-237-9292
British Airways	800-247-9297
Continental	800-525-0280
Delta	800-221-1212
Japan Airlines	800-525-3663
Jet Blue	800-538-2583
KLM	800-374-7747
Lufthansa	800-645-3880
Northwest	800-225-2525
United	800-241-6522
US Airways	800-428-4322