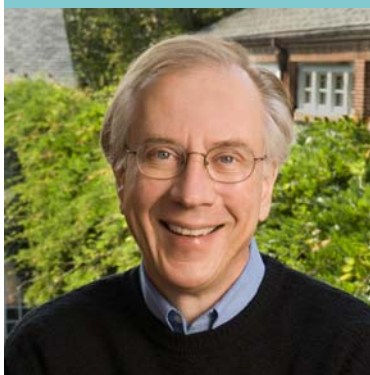


University of Colorado Denver

Postdoctoral Research Day

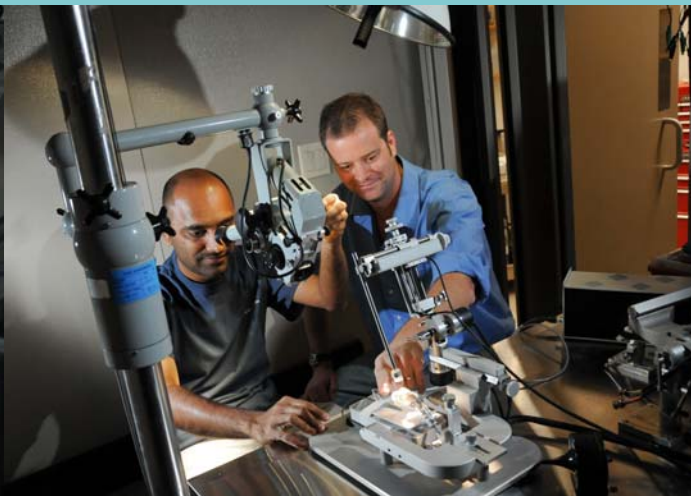
Fri., May 14, 2010



Keynote speaker: Dr. Thomas R. Cech
Nobel Laureate in Chemistry, 1989
Distinguished Professor, UC-Boulder



Featuring: Dr. Rodney Ulane
NIH Training Officer and Director
Division of Scientific Programs at OER



POSTDOCTORAL RESEARCH DAY 2010

MAY 14, 2010

AGENDA

TIME	EVENT	LOCATION
8:30am	Information tables open: Alternatives in Sciences Club, CCTSI, Health Sciences Library, Grants & Contracts, University Bookstore, Campus Police	1 st floor, Research 1N
9:00am	Welcome: Richard Traystman, Ph.D. Vice Chancellor for Research	Hensel Phelps auditorium Research 1N
9:05am	Workshop Presentation: “NIH Grants for the Development of Your Research Career: Understanding the NIH Grants Process and How to Navigate It” Rodney Ulane, Ph.D. Training Officer and Director, Office of Extramural Programs, NIH	
9:30am	Break	
9:40am	Workshop Participation Session	
10:45am	Break	1 st floor, Research 1N
11:00am	Keynote Address: “How to Win a Nobel Prize” Thomas Cech, Ph.D. Nobel Laureate, Professor of Chemistry, University of Colorado	Hensel Phelps auditorium Research 1N
12:00pm	Lunch (on your own)	
1:00pm	Poster and judge check in/assignments	2 nd floor, Research 2
1:30pm	Poster Session 1/judging	
2:30pm	Poster session 2/judging	
3:30pm	Reception	Room 2100, Research 2
4:00pm	Comments – Provost Rod Nairn, Ph.D. Award presentation – Dean John H. Freed, Ph.D. Introduction of the UCD-Postdoctoral Association Executive Council Poster session awards announced	



University of Colorado Denver
Postdoctoral Office

www.ucdenver.edu/postdoc

WELCOME

Postdoctoral Research Day

14 May 2010

It is my pleasure to welcome you to the first Postdoctoral Research Day at the University of Colorado Denver. This day is intended to highlight the contributions of the over 300 postdocs at the University of Colorado Denver, and our affiliated institutions.

Although relatively small in numbers, postdocs contribute disproportionately to the University community, both through their many contributions to the research enterprise and by their creativity and energy in the pursuit of knowledge. Postdocs are also the next generation of research scientists, whether it be in academia or in industry.

The planning committee for Postdoctoral Research Day thought that there would be no better way to acknowledge the many contributions of the postdocs at our institution than to offer a day combining career development with an opportunity for the postdocs to showcase their current research efforts. Thus, the morning is being devoted to career development, with talks by Dr. Rodney Ulane, Training Officer and Director of the Division of Scientific Programs at the NIH, and Dr. Tom Cech, Nobel Laureate in chemistry and Director of the Colorado Initiative in Molecular Biotechnology; the afternoon will be a poster session where over 70 of our postdocs will present their work.

I hope you enjoy all aspects of the day and will take the opportunity to talk to the postdocs about their research and career aspirations.

I want to close by thanking all those who made the day possible – from the sponsors and donors, to the planning committee and the Postdoctoral Association leadership.

Best wishes,

John Freed

John H. Freed, Ph.D.
Professor of Immunology
Dean of the AMC Graduate School
University of Colorado Denver

PDRD 2010 PLANNING COMMITTEE MEMBERS

Carol Aherne	Postdoctoral Fellow, Dept. of Anesthesiology, SOM UCD-Postdoctoral Association Executive Council
John Freed	Dean, Graduate School, Anschutz Medical Campus
Erin Giles	Postdoctoral Fellow, Dept. of Medicine, Div. of Endocrinology, Metabolism & Diabetes, SOM Postdoctoral Advisory Committee, UCD-PDA Executive Council
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Valerie Saltou	Postdoctoral Office Coordinator
John Sladek	Professor, Depts. of Pediatrics and Neurology, SOM Postdoctoral Advisory Committee

SPECIAL THANKS go to these postdocs for their significant contributions to the success of the day:

Heidi Grabbenstatter	Postdoctoral Fellow, Dept. of Pediatrics, SOM UCD-Postdoctoral Association Executive Council
Ryan Mays	Postdoctoral Fellow, Dept. of Medicine, Div. of Cardiology, SOM UCD-Postdoctoral Association Executive Council
Enrique Torchia	Postdoctoral Fellow, Dept. of Dermatology, SOM Postdoctoral Advisory Committee, UCD-PDA Executive Council

THE POSTDOCTORAL RESEARCH DAY 2010
has been made possible by generous contributions from:

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NETRIN-1 AT THE INTERFACE OF MUCOSAL HYPOXIA AND INFLAMMATION

Carol M. Aherne¹, Peter Rosenberger², Colm B. Collins³, Jesús Rivera-Nieves³, Holger K. Eltzschig^{1,2}.

¹Mucosal Inflammation Program, Department of Anesthesiology, University of Colorado Denver, Aurora, CO, USA; ²Department of Anesthesiology and Intensive Care Medicine, Tübingen University Hospital; ³Mucosal Inflammation Program, Department of Gastroenterology, University of Colorado Denver, Aurora, CO, USA.

Categorized as inflammatory bowel diseases (IBD), Crohn's disease and ulcerative colitis are chronic inflammatory conditions of the intestinal mucosa that affect more than one million Americans. Intestinal inflammation, as experienced in IBD, is associated with profound tissue hypoxia, with the mucosal surface being particularly prone to inflammation-associated hypoxia. Recent evidence suggests a protective role of hypoxia-elicited signaling pathways in attenuating intestinal inflammation. Based on the notion that the neuronal guidance molecule netrin-1 inhibits leukocyte transmigration *in vitro* and *in vivo*, we pursued its role in experimental IBD at the interface between hypoxia and inflammation. Preliminary studies presented here demonstrate hypoxia induction of netrin-1 expression and its secretion from intestinal epithelial cells. Further analysis revealed netrin-1 attenuation of hypoxia-induced mucosal inflammation through adenosine-dependent signaling pathways. Functional studies suggested netrin-1 attenuation of neutrophil transepithelial migration, while mice with a partial genetic deletion of netrin-1 demonstrate increased inflammatory cell accumulation and higher systemic cytokine levels during hypoxia exposure (8h, 8% oxygen). Finally, preliminary studies in mice with chemically induced colitis indicate netrin-1 induction during experimental colitis, in conjunction with a less severe disease activity following exogenous netrin-1 treatment. Taken together, these studies present netrin-1 as an endogenous mediator for the attenuation of hypoxia-induced mucosal inflammation.

PROTEIN PROFILING IN MOUSE MODEL OF DOWN SYNDROME

MM Ahmed, D Dubach, and K Gardiner, Department of Pediatrics, Intellectual and Developmental Disability Research Center, Neuroscience and Human Medical Genetics Programs, University of Colorado, Denver, CO.

Down syndrome (DS) is the most common genetic cause of intellectual disability. It is caused by an extra copy of human chromosome 21 (HSA21) and the increased expression, due to gene dosage, of the genes it encodes. This overexpression is predicted to cause widespread perturbations in protein profiles in brain and other tissues. To identify potential targets for pharmacotherapies it is necessary to characterize these perturbations. Here we describe results of protein profiling in two mouse models of DS, the TC1 (Transchromosomal) model, which is trisomic for ~100 HSA21 genes, and the Ts65Dn model, which is trisomic for a partially overlapping set of 89 HSA21 genes. We are using quantitative Western blots and Reverse Phase Protein Arrays to assay levels and dynamic changes in phosphorylation and localization of key proteins involved in NMDAR, MAP kinase and calcineurin signaling and apoptosis pathways. In the course of this work, we identified patterns in protein phosphorylation that are sensitive to methods of protein lysate preparation that include use of heat and kinase inhibitors to inactivate enzymes involved in protein modification.

This work was funded by NICHD & The Foundation Jerome Lejeune

BLOCKADE OF CD40 SIGNALING ON DIABETOGENIC CD4 T CELLS INHIBITS EFFECTOR FUNCTION

Rocky L. Baker, Thierry Mallevaey, Laurent Gapin, and Kathryn Haskins
National Jewish Health,
University of Colorado, Denver, CO.

IFN-gamma-secreting Th1 T cells play a pivotal role in the development of type 1 diabetes. We previously reported that diabetogenic Th1 T cell clones express the costimulatory molecule CD40 and that treatment of these cells with an antibody to CD40 abrogates their capacity to transfer disease, indicating that engagement of CD40 on T cells contributes to their diabetogenicity. To further investigate the effect of CD40 blockade on effector function of Th1 cells, we produced a variant of a diabetogenic T cell clone BDC-5.2.9 expressing a dominant-negative (DN) form of the CD40 molecule. When BDC-5.2.9 was transduced with CD40DN, the T cells produced only very low levels of IFN-gamma when challenged with antigen in vitro, suggesting that signaling through the CD40 molecule is necessary for effector function. BDC-5.2.9 is highly diabetogenic in young NOD or NOD.scid recipient mice and induces full-blown disease within a two-week period following injection. When the CD40DN variant of BDC-5.2.9 was tested in vivo, none of the recipients developed diabetes. The demonstration that effector function of pathogenic T cells is inhibited through expression of a non-functional CD40 molecule on the T cells suggests a signaling requirement through CD40. Since diabetogenic T cells can co-express both CD40 and its ligand CD154 upon activation, it is possible that their pathogenic properties are enhanced through T-T interactions via CD40-CD154. We conclude from these experiments that blocking CD40 on autoreactive T cells alters their effector function and hypothesize that engagement of CD40 on Th1 T cells promotes diabetogenicity.

MAPPING T CELL RECEPTOR RECOGNITION OF A BERYLLIUM ANTIGEN WITH IMPLICATIONS FOR CHRONIC BERYLLIUM DISEASE

NA Bowerman¹, MT Falta¹, J Kappler², AP Fontenot^{1,2}

¹Department of Medicine, University of Colorado Denver, Aurora, CO

²Integrated Department of Immunology, National Jewish Health, Denver, CO

Exposure to the metal beryllium in the workplace can result in chronic beryllium disease (CBD), which is associated by the accumulation of large expansions of beryllium-specific CD4⁺ Th1 cells in the lung. Several T cell clones have been isolated from the lung of a CBD patient that recognize beryllium in the context of HLA-DPB1*0201/peptide. Sequence comparison of the TCR complementarity determining regions (CDRs) has revealed nearly identical V β and CDR3 V α domains, yet different CDR1-2 V α regions (due to expressing different TCRAV gene segments). Hence, we propose an atypical docking footprint for recognition of the beryllium antigen by these specific TCRs, with CDR1 α and CDR2 α not significantly contributing to T cell recognition. To test this hypothesis, we have focused on a particular V α 22⁺/V β 5⁺ beryllium-specific TCR derived from a T cell clone designated JK28. Single-site alanine substitutions have been introduced across the CDRs of this TCR in order to delineate amino acids that are critical for recognition of the HLA-DP2/Be²⁺/peptide complex. TCR variants containing alanine substitutions were expressed on a murine $\alpha\beta$ T cell hybridoma also transduced with human CD4. Assays comparing IL-2 secretion when T cell hybridomas containing wildtype and mutated TCRs are stimulated with beryllium support the notion that the V β region contributes most to antigen recognition by this TCR.

This work was funded by NIH, HL062410 and HL092997.

**EFFECT OF ODOR EXPOSURE ON
GLOMERULAR SIZE IN THE MOUSE
OLFACTORY BULB**

Nicolas Busquet, Josephine Todrank, Giora Heth and Diego Restrepo.
University of Colorado, Denver, CO.

Glomeruli in the olfactory bulb are formed by the coalescence of olfactory sensory neurons (OSNs) expressing the same receptor in the olfactory epithelium. Glomeruli vary in size depending on the number of OSNs projecting to them. A previous study indicated that pairing odor exposure with aversive stimuli increased the size of GFP-tagged glomeruli with OSNs that were activated by the odor. Shaping the olfactory system in response to changes in the environment could have evolutionary benefits, such as heightened sensitivity to odors of available, palatable foods. In this pilot study, we explored the effects of either eating or simply smelling a scented diet on glomerular size in male and female adult mice. Genetically modified mice expressing GFP in the M71 receptors were exposed to the target odorant, acetophenone, for 3 weeks. The volume of tagged glomeruli (estimated from areas of serial 20 μm sections) revealed significant effects of odor exposure on glomerular size. Medial, but not lateral, glomeruli of exposed animals were significantly larger than those of control animals. Glomeruli were larger in females than males, irrespective of their body weight. A more extensive study will be necessary to determine the perceptual and behavioral implications of these differences.

This work was funded by NIH F33DC009137 (J.T), DC04657 (D.R.) and DC006070 (D.R)

KNOCKDOWN OF HPRT ENABLES SELECTION OF GENETICALLY MODIFIED HUMAN HEMATOPOIETIC PROGENITOR CELLS IN VITRO: IMPLICATIONS IN GENE THERAPY.

Rashmi Choudhary and Christopher Porter,
Department of Pediatrics-
Hematology/Oncology, University of Colorado
Denver, CO 80045.

Purpose of study: Hematopoietic stem cells (HSCs) are potential targets for many applications of gene therapy, owing to their ability to differentiate into all lineages of the hematopoietic system. However, HSC gene therapy has shown limited clinical efficacy because of the small proportion of engrafted genetically corrected HSCs. The use of drug-resistance genes to enable selection for transduced HSCs has been explored, but with limited success. Previous studies from our lab indicated that resistance to 6TG, a relatively non-toxic drug, could be provided to cells using shRNA against hypoxanthine-guanine phosphoribosyltransferase (HPRT). Moreover, this was sufficient for *in vitro* and *in vivo* selection of transduced murine hematopoietic progenitor cells. We aim to use this approach for *in vivo* selection of genetically corrected hematopoietic cells to cure human diseases.

Methods used: Human CD34+ cells (HSC enriched population) were isolated from cord blood using CD34 Positive selection kit as per the instructions. Human Acute Myeloid Leukemia (AML) (Molm13, MV4-11) and Acute lymphoid leukemia (ALL) cell lines (Reh) were cultured in RPMI media. Using lentiviral vectors, cells were transduced with two shRNA constructs (491 and 50) targeting HPRT. Cells were cultured in the absence or presence of different doses of 6TG. Live cell concentrations and percentages of GFP+ cells were determined by flow cytometry.

Summary of results: Real-Time and western blotting data demonstrated that construct 491 was most efficient in knocking down HPRT in human AML and ALL cell lines compared to construct 50. While control transduced cells decreased in numbers with increasing concentrations of 6TG, cells transduced with constructs 491 and 50 were resistant to 6TG. Construct 491 was most effective in providing 6TG resistance with relatively higher IC50

values compared to 50. Based on these results, 491 was used to transduce human CD34+ progenitor cells. Similar to the effects in cell lines, proliferation of control transduced CD34+ cells diminished in response to 6TG treatment, in a dose dependent manner, while 491 transduced cells remained resistant to the effects of 6TG. This was associated with an increase in the percentage of GFP+ cells in 491 transduced group (28% increase) compared to control group, indicating a selective advantage conferred to 491 transduced cells in the presence of 6TG.

Conclusions: Our results indicate that interference RNA mediated purine analog resistance provides a selective advantage for human hematopoietic progenitor cells in the presence of 6TG. Subsequent studies will be performed in xenograft models of human hematopoiesis.

This work was funded by 1R21HL094921-01A1.

HEPATITIS C VIRUS (HCV) ASSOCIATED STEATOSIS, INSULIN RESISTANCE AND INCREASED GLUCONEOGENIC GENE EXPRESSION IN HUH8 CELLS: ESSENTIAL ROLE OF NS5A AND C/EBP β .

M Choudhury¹, I Qadri^{1*}, M Rahman¹, TA. Knotts¹, RC. Janssen¹, M Iwahashi³, L Puljak⁴, FR. Simon³, G Kilic⁴, GJ. Fitz⁴ and JE. Friedman^{1,2,1} Department of Pediatrics, ²Department of Biochemistry and Molecular Genetics, ³Division of Gastroenterology and Hepatology, Department of Medicine, University of Colorado at Denver and Health Sciences Center, Denver CO, 80045, ⁴Department of Internal Medicine, University of Texas Southwestern Medical Center, Dallas, TX 75390.

Chronic infection with hepatitis C virus (HCV) is a worldwide epidemic with an incidence rate of more than 600 million affected individuals. HCV greatly increases the risk for type II diabetes and steatohepatitis; however the cellular mechanisms remain poorly understood. Here we utilized a Huh7 derived cell line engineered with the stably infected replicon for HCV, termed Huh8, and one lacking the key non-structural element NS5A. We found that transcription of the key gluconeogenic enzyme *PEPCK* and associated transcription factors are dramatically up-regulated in Huh.8 cells, and responded robustly to NS5A expression/deletion. Maximal induction of *PEPCK* involved activation of the CRE element in the *PEPCK* promoter. HCV induced an increase in activation of cyclic adenosine monophosphate response element binding (CREB), CCAAT/Enhancer Binding Protein β (C/EBP β), Foxo1, and Peroxisome Proliferator Gamma Co-Activator-1 (PGC1 α). Transduction of recombinant adenovirus encoding dominant negative CREB or C/EBP β RNAi in Huh.8 cells significantly reduced or normalized *PEPCK* gene expression, with no change in PGC1 α or Foxo1 levels. HCV-replicating cells also demonstrated increases in cellular lipids with insulin resistance at the level of the insulin receptor, increased 307Ser-IRS-1, and decreased 473-Akt activation in response to insulin. Expression of the HCV nonstructural component NS5A in Huh7 or primary hepatocytes stimulated *PEPCK* gene expression, while a deletion in NS5A in the subgenomic replicon greatly reduced *PEPCK*

gene expression and cellular lipids, but was without effect on insulin resistance as demonstrated by the inability of insulin to mobilize membrane vesicles to the plasma membrane. Insulin resistance was accompanied by increased expression of lipogenic genes sterol regulated element binding protein-1c (SREBP-1c), liver X receptor (LXR), and stearoyl-Coenzyme A desaturase-1 (SCD-1) and increased lipid export genes MTP and APOB-100. C/EBP β RNAi reduced expression of lipogenic genes but was unable to reduce the accumulation of triglycerides in HCV cells, suggesting dissociation between lipogenesis and lipid retention in Huh8 cells. We conclude that HCV-induced NS5A and C/EBP β is partially responsible for the NASH like features in Huh8 cells, but that chronic TG accumulation may involve other mechanisms. Collectively, these data reveal an important role of NS5A, C/EBP β and pCREB in promoting HCV-induced lipid accumulation and gluconeogenic gene expression and suggests cellular mechanisms other than NS5A are involved in the mechanism(s) for HCV-induced lipid retention and steatosis.

This work was supported by a grant from American Liver Foundation (Herman Lopeta Memorial Award to I.Q.) and grant R01-DK59767 from the National Institutes of Health (to J.E.F.).

DOWNREGULATION OF MIR-342 IS ASSOCIATED WITH TAMOXIFEN RESISTANT BREAST TUMORS

Cittelly DM¹, Spoelstra NS¹, Edgerton SM¹, Richer JK¹, Thor A, Jones FE². ¹University of Colorado, Denver, CO. ²Tulane University, New Orleans, LA

Tumor resistance to the selective estrogen receptor modulator tamoxifen remains a serious clinical problem especially in patients with tumors that also overexpress HER2. We have recently demonstrated that the clinically important isoform of HER2, HER2 Δ 16, promotes therapeutically refractory breast cancer including resistance to endocrine therapy. Likewise additional breast tumor cell models of tamoxifen resistance have been developed that do not involve HER2 overexpression. However, a unifying molecular mechanism of tamoxifen resistance has remained elusive. Here we analyzed multiple cell models of tamoxifen resistance derived from MCF-7 cells to examine the influence of miRNAs (miRs) on tamoxifen resistance. We compared miR expression profiles of tamoxifen sensitive MCF-7 cells and tamoxifen resistant MCF-7/HER2 Δ 16 cells. We observed significant and dramatic downregulation of miR-342 in the MCF-7/HER2 Δ 16 cell line as well as the HER2 negative but tamoxifen resistant MCF-7 variants TAMR1 and LCC2. Restoring miR-342 expression in the MCF-7/HER2 Δ 16 and TAMR1 cell lines sensitized these cells to tamoxifen-induced apoptosis with a dramatic reduction in cell growth. Expression of miR-342 was also reduced in a panel of tamoxifen refractory human breast tumors, underscoring the potential clinical importance of miR-342 downregulation. Towards the goal of identifying direct and indirect targets of miR-342 we restored miR-342 expression in MCF-7/HER2 Δ 16 cells and analyzed changes in global gene expression by microarray. The impact of miR-342 on gene expression in MCF-7/HER2 Δ 16 cells was not limited to miR-342 in silico predicted targets. Ingenuity Pathways Analysis of the dataset revealed a significant influence of miR-342 on multiple tumor cell cycle regulators. We found a highly significant association of miR-342 with cell apoptosis, consistent with our observation that ectopic miR-342 expression sensitized tamoxifen

resistant cells to tamoxifen-induced apoptosis. miR-342 expression alone is not sufficient to induce cell death, but miR-342 sensitizes cells to apoptosis associated with estrogen-deprivation and tamoxifen exposure. In this context, the miR-342 indirect target thioredoxin-interacting protein (TXNIP) is the most dramatically upregulated gene in response to miR-342 expression and could potentially mediate miR-342 action. Deciphering the interplay between miR-342 and TXNIP is currently in progress. Our findings suggest that miR-342 may emerge as an important tumor marker predicting tamoxifen response in breast cancer patients. Since miR-342 regulates expression of genes involved in tamoxifen mediated tumor cell apoptosis and cell cycle progression, restoring miR-342 expression may represent a novel therapeutic approach to sensitizing and suppressing the growth of tamoxifen refractory breast tumors. This work was funded by Department of Defense concept Award to DC.

CONTROL OF DICER BY MICRORNAS

DR Cochrane, E McKinsey, DM Cittelly, NS Spoelstra, A Elias, AC Tan and JK Richer, Department of Pathology, University of Colorado Denver

Dicer catalyzes the last step in miRNA biosynthesis which generates the fully active, mature miRNA. There is emerging evidence that miRNAs are particularly important for maintenance of an epithelial phenotype and there are lower miRNA levels in stem cells and aggressive cancer cells that have undergone epithelial to mesenchymal transition (EMT). Low Dicer levels can result in decreased miRNA levels. Indeed, Dicer is lower in breast cancer cell lines and clinical samples that have undergone EMT and functions as a haploinsufficient tumor suppressor. To identify additional miRNAs associated with EMT, we performed miRNA profiling of estrogen receptor alpha (ER) positive luminal A (MCF7 and T47D) versus ER- triple negative breast cancer (TNBC) cell lines (MDA-MB-231 and BT549). Consistent with previous studies, we find that many more miRNAs are lower in the aggressive TNBC cells than are gained. However some miRNAs are higher in TNBC, such as miR-222/221, which are over 90 fold higher in TNBC cell lines and are only expressed in ER negative clinical specimens as these miRNAs target ER. Interestingly miR-222/221 and miR-29 (the top miRNAs more abundant in TNBC cells) are predicted to directly target Dicer itself. We therefore manipulated the levels of these miRNA in breast cancer cells to determine if they affect Dicer protein levels. Addition of miR-221, miR-222 or miR-29 mimics T47D luminal A cells dramatically reduces the levels of Dicer protein. Conversely, antagomiRs of miR-221/222 increase Dicer levels in MDA-MB-231 cells. Using a luciferase reporter assay, we demonstrate that Dicer is directly targeted by miR-221/222 and miR-29a. Additionally, when miR-200c, termed the "guardian of the epithelial phenotype," is restored to TNBC cells that have undergone EMT, Dicer protein expression is increased to levels observed in luminal A cells; however, we believe that this occurs via an indirect mechanism. Our data indicate that miR-221/222 controls the aggressive behavior of TN breast cancers by simultaneously repressing ER and Dicer itself, and that two of the other most differentially expressed miRNA in TN

versus luminal A breast cancers (miR-29a and miR-200c) also affect Dicer levels. Since low Dicer levels are associated with aggressive cancers, we can envision miRNA-based treatments that serve to increase Dicer levels and therefore alter the global miRNA expression patterns. This may have potential for targeted treatment of TN breast cancers for which there are currently no optimal therapeutics.

This work was funded by DOD BC084162 to JKR

**DEFICIENT RETINOIC ACID SYNTHESIS BY
INTESTINAL CD103⁺ DENDRITIC CELLS
TRIGGERED EPITHELIAL COMPENSATION
IN CROHN'S-LIKE MURINE ILEITIS.**

Colm B. Collins¹, Carol M. Aherne¹, Eóin N. McNamee¹, Matthew D.P. Lebsack¹, Paul Jedlicka², Holger K. Eltzschig¹, Jesús Rivera-Nieves¹
¹Mucosal Inflammation Program,
²Department of Pathology, University of Colorado, Denver, Colorado 80045.

The enteric immune system exists in a permanent semi-inflamed state, poised to respond to pathogens, while remaining refractory to non-pathogenic antigens. This balance is maintained through complex interaction of immune cells including epithelial (EC), dendritic (DC) and T cells. Production of RA by the former cell types drives induction of regulatory T cells, preventing inappropriate immune activation, as seen in Crohn's disease (CD). By using a TNF-driven model of Crohn's-like ileitis (i.e. B6.129P-TNF \square AUrich element [TNF \square ARE]) that recapitulates many features of CD, we observed that inflamed ilea displayed greater frequency of DC with an activated phenotype, whereas pro-regulatory CD103⁺ DC decreased in frequency during advanced disease. Furthermore CD103⁺ DC from inflamed mice exhibited decreased expression of retinoic acid machinery (RALDH2), while the proportion of CD4⁺ CD25⁺ Foxp3⁺ regulatory T cells increased nonetheless. Intestinal epithelial cells responded to decreased DC RALDH2 transcripts with up-regulation of RA machinery both *in vivo* and *in vitro*. The attempt by EC to offset RA synthetic deficiency of DC was clearly insufficient to attenuate disease. However, supplementation with RA significantly attenuated disease through expansion of regulatory T cells and reduction of Th17 cells. In summary, our findings support an important role for RA synthetic deficiency in perpetuating chronic murine ileitis mediated by TNF over-production.

Funded by NIH-1R01DK080212-01A2 and CCFA-2652

ACTIVATION CHARACTERISTICS OF SURFICIAL AND FINE WIRE EMG DURING FLEXION-RELAXATION OF THE LUMBAR SPINE

BS Davidson, BA Enebo, and M Solomonow, Department of Orthopaedics, The Center for Integrative Medicine, University of Colorado, Denver, CO.

Surface EMG recordings of the erector spinae group (ES) have frequently been used as a surrogate for multifidus (MF) activation in biomechanical investigations. However, a direct comparison has not been performed to justify this assumption. Therefore, the objective of the investigation was to examine differences in activation timing between surface EMG recordings of the ES and fine wire EMG recordings of the MF.

Fourteen volunteers performed flexion-extension trials consisting of 3 sec flexion from neutral standing, 3 sec pause in deep flexion, and 3 sec extension back to neutral standing. 3D orientation of the torso and pelvic segments were defined by reflective markers affixed to bilateral ASIS and PSIS, C7, T10, and sternum. Lumbar flexion angle was defined as the torso angle projected onto the sagittal plane of the pelvic coordinate system.

Following routine EMG skin preparation and subsequent sterilization, 50 μ m fine wire pairs were inserted bilaterally into the MF at the L3/L4 level. Ag-AgCl surface electrodes were placed above the ES with an interelectrode distance of 4cm to accommodate for the fine-wire recordings. Raw EMG was processed using the linear envelope method (10Hz LP cutoff for activation timing, 5Hz LP cutoff for amplitude measures). Threshold of activation onset and termination was set as 10% of maximum amplitude for each trial. Muscle activation timing was expressed as the lumbar flexion angle normalized to maximum flexion angle of the trial. Differences between muscles were assessed using mixed model statistics. ES and MF amplitudes were compared using correlation coefficients, and a time-course of the median frequency during the active periods were calculated using 0.5 sec epochs spaced at an interval of 0.05 sec.

EMG profiles demonstrated a typical flexion-relaxation response with termination of the signals after reaching full flexion and onset of the signals to facilitate extension movement.

Activations normalized to lumbar flexion angle indicated that the ES had 4.1% later termination during the flexion phase ($p=0.008$) and 1.5% earlier onset in the extension phase ($p=0.032$) than MF. Amplitude characteristics had similar trajectories and correlation coefficients ranging from 0.2-0.6 (low correlation). Throughout the range of movement, spectral response of MF was relatively constant while the ES varied proportionally with lumbar flexion angle.

In summary, the results indicate differences in EMG response of the two muscles. Methodological concerns regarding a direct comparison of spectral characteristics because two modalities were used. However, it is apparent that MF activity cannot be characterized using surface electrodes.

This research was funded by National Institutes of Health (K99 AT004983-01A1-09)

STRUCTURAL STUDIES OF EARLY B CELL FACTOR (EBF), AN ESSENTIAL REGULATOR OF B CELL DEVELOPMENT

KJ Decker, J Hagman, and ME Churchill, Affiliations, University of Colorado, Denver, CO and National Jewish Health, Denver, CO.

Early B cell Factor (EBF) is an essential regulator of genes in B lymphocytes. EBF drives the development of early progenitors to become B cells by activating genes encoding the pre-B and mature B cell receptors. The purpose of this study is to determine the three-dimensional structure of murine Ebf bound to DNA. We will use X-ray crystallography to gain new structural information on EBF's domain structure, dimerization interfaces, and potential roles for individual amino acids and elements of secondary structure in EBF function. This structure will reveal the architecture of the DNA binding and dimerization domains which may include a novel zinc-coordination motif, and will aid in understanding how EBF interacts with other proteins (e.g. Pax5 and Runx1). Finally, the structure will also allow us to determine the basis for EBF binding to palindromic and non-palindromic DNA fragments, such as those based on consensus sequences found in physiologically-relevant promoters (including the mb-1 promoter).

This work was funded by the NIH.

A PROTEOMIC STRATEGY REVEALS CHROMOGRANIN A AS THE ANTIGEN TARGET FOR HIGHLY DIABETOGENIC CD4+ T CELL CLONES

T DeLong, BD Stadinski, N Reisdorph, R Reisdorph, J Kappler and K Haskins, University of Colorado, Denver, CO.

Pathogenesis of type 1 diabetes (T1D) is mediated by autoreactive T cells directed toward antigens in islet beta cells. Several candidate antigens such as insulin and GAD have been much investigated, but it is not known if these are the key autoantigens that drive pathogenic T cells. We have used a biochemical and proteomic approach to identify the beta cell autoantigens for a panel of diabetogenic CD4 T cell clones derived from NOD mice. The BDC-2.5 clone is especially well-known because of the BDC-2.5 TCR-transgenic mouse, and mimotopes for this clone have been previously described. Two other diabetogenic T cells, BDC-5.10.3 and BDC-10.1, exhibit overlapping responses to the BDC-2.5 peptide mimotopes and to biochemically separated fractions. We isolated beta cell tumors from NOD RIP-TAg mice and separated fractions from tumor cells by differential centrifugation followed by sequential size exclusion and ion exchange chromatography. Antigenic fractions from the chromatographic separation were analyzed by mass spectrometry. Six protein candidates were identified, including three members of the secretogranin family and insulin. Only one candidate, chromogranin A, showed homology to BDC-2.5 peptide mimotopes. Identification of the antigen as chromogranin A was confirmed by discovery of a natural sequence that can stimulate all three T cell clones and by demonstrating that islets from chromogranin A-deficient mice are without antigenic activity. These findings indicate that the natural ligand for three diabetogenic T cell clones, all isolated from different mice, is a peptide of the secretory granule protein chromogranin A.

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REVERSE PHASE PROTEIN ARRAYS TO IDENTIFY PROTEIN PATHWAY PERTURBATIONS IN DOWN SYNDROME

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Down syndrome (DS), resulting from an additional copy of human chromosome 21 (HSA21), is the most common genetic cause of intellectual disability. Due to the large number of genes overexpressed in DS (HSA21 encodes ~200 confirmed protein coding genes), it is likely that numerous pathways and cellular processes are affected. In order to identify the most critical proteins whose expression levels, phosphorylation status or subcellular localizations are altered due to trisomy 21, we have been optimizing the reverse phase protein array (RPPA) technology. As well as screening lysates from brain regions of mouse models of DS, we have recently embarked upon a RPPA project utilizing B-lymphoblastoid cell lines. We obtained eight DS and eight control cell lines and carried out cellular fractionations before generating a large scale RPPA. Slides are screened with subsets of proteins encoded by HSA21 and non-HSA21 proteins functioning in pathways predicted to be altered by trisomy, including components of MAP kinase and calcineurin signaling, apoptosis and oxidative stress pathways. We expect to gain novel insight into various protein pathway alterations in DS, assess the inter-individual variation in these alterations and predict additional perturbations that may exist in the DS brain. .

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USP18 REGULATES EGFR AND CANCER CELL SURVIVAL VIA miR-7

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Elevated expression of the proto-oncogene epidermal growth factor (EGF) receptor (EGFR) is associated with many types of cancer. Identifying regulators of EGFR is important to the ongoing efforts of developing successful strategies to combat the high levels of EGFR found in many types of tumors. Past studies from our lab identified the ubiquitin specific protease Usp18 as a novel, potent regulator of EGFR. Interestingly, Usp18 regulation of EGFR occurs at the 3'UTR of EGFR mRNA. Recent literature has shown that the microRNA miR-7 also regulates EGFR expression at the 3'UTR. The data presented here show that depletion of Usp18 from numerous cancer cell lines leads to a dramatic increase in miR-7 levels and activity and a corresponding decrease in the levels of EGFR. These changes can be reversed with the addition of a miR-7 inhibitor. Furthermore, we show that loss of Usp18 from cancer cells leads to increased caspase 3/7 activity and increased apoptosis. Finally, the invasiveness of numerous cancer cell lines is also reduced upon depletion of Usp18. Taken together the studies presented here demonstrate that inhibition of Usp18 in numerous cancer cell lines reduces the levels of the proto-oncogene EGFR and reduces the invasiveness of cancer cells by promoting apoptosis.

GREY PLATELET SYNDROME: THE HUNT FOR THE DISEASE CAUSING GENE.

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Platelets are generated in the bone marrow by megakaryocytes and have multiple roles in thrombosis and hemostasis. Platelets form the initial hemostatic plug at the site of blood vessel injury, and also release a number of molecules that regulate hemostasis, and induce tissue growth and repair at the site of vessel injury. Grey platelet syndrome (GPS) is a rare bleeding disorder that affects megakaryocytes and platelets. Patients with GPS present with mild to moderate thrombocytopenia and very large platelets that lack α -granules. When a platelet becomes activated, the α -granules release their contents which are involved in several aspects of normal platelet function. The absence of these granules leads to decreased clotting ability, therefore, patients with GPS present with mild to severe bleeding. Elucidating the genetic cause of GPS will aid physicians in proper treatment of patients with this disease as well as aid researchers in further delineating the complex pathways that lead to α -granule formation. In this study, we analyzed two Native American families with autosomal recessive inheritance of GPS. Wright-Giemsa blood smears as well as electron micrographs were taken of affected patients to confirm the large size of the platelets as well as the absence of the α -granules. Obligate heterozygotes do not exhibit a platelet phenotype. DNA was extracted from whole blood. The affected patients as well as unaffected parents and siblings were genotyped using the Affymetrix 6.0 one million SNP chips. These data were analyzed for regions of homozygosity using the Affymetrix Genotyping Console software. We found two regions of homozygosity that were shared between all of the affected individuals on chromosomes 1q25.1 and 3p21. These regions were examined for genes that play a role in binding to proteins of the cytoskeleton, protein transport, vesicle transport, and regulation of transcription, all potential processes involved in alpha granule formation. Several promising candidate genes for mutation analysis were found using the criteria described above.

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SIRT3 INHIBITION BY 4-HYDROXYNONENAL CHARACTERIZED BY MALDI-TOF/TOF AND MOLECULAR MODELING STUDIES

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Alcoholic liver disease (ALD) is a major cause of morbidity and mortality in the Western world. A recently discovered effect of chronic ethanol consumption is a marked increase in hepatic protein acetylation. While hepatic mitochondrial dysregulation is a known consequence of ethanol ingestion, the underlying mechanisms behind this remain elusive. Furthermore, increases in oxidative stress and lipid peroxidation are well documented, resulting in the accumulation of reactive aldehydes such as 4-hydroxynonenal (HNE). HNE has been shown to play a role in cell signaling and, at excesses, may perturb normal physiological processes. SIRT3, a mitochondrial deacetylase, regulates global mitochondrial protein acetylation and alterations in its activity due to ethanol metabolism may play a key role in the mitochondrial dysfunction associated with ALD. Modulations in SIRT3 deacetylase activity resulting from ethanol metabolism and HNE adduction was investigated *in vitro* via HNE exposure and mass spectrometry (MS). Additionally, a mouse model of chronic ethanol consumption was utilized to elucidate underlying mechanisms of altered mitochondrial protein acetylation.

INVOLVEMENT OF WNT/BETA-CATENIN SIGNALING IN THE RENEWAL OF MATURE TASTE BUD OF MICE

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Taste buds are onion shaped structures composed of three different cell types. Type I are thought to be glial-like cell acting as support cell. Type II cells express the apical receptors and the transduction proteins involved in the detection of sweet, bitter and umami. Type III are sour detector cells and establish conventional connections with afferent synapses. Renewal of the three different cell types within taste buds occurs about every 10-14 days. The molecular mechanisms responsible for this turnover are not completely elucidated. Embryonic taste bud development has been shown to be controlled by Wnt/ β -catenin signaling (Liu *et al.*, 2007). Thus, the present work aims at investigating whether this pathway is involved in taste cell renewal in adult mice. Using the BATGAL strain which expresses β -galactosidase (β -gal) in the presence of nuclear β -catenin, we first examined what cell types are Wnt responsive by performing immunohistochemistry. Quantifying double labelling for cell type markers and β -gal showed that Wnt/ β -catenin signaling is mainly active in NTPDase2 (type I) positive cells in circumvallate papillae. Less frequently, β -gal was detected in α -gustducin (type II) and NCAM (type III) positive cells. Then, mice conditionally overexpressing Dkk1, a secreted Wnt inhibitor, were used to explore the functions of Wnt/ β -catenin pathway in taste buds. These mice carrying tetracycline-dependent Dkk1 alleles were fed doxycycline chow to induce transgene expression. Two-bottle tests between water and different concentrations of denatonium revealed no

difference in the preference ratio curve between control mice and Dkk1-overexpressing mice after 2 and 6 weeks of drug treatment. Nevertheless, because our preliminary data showed a shift in the preference curve after 9 months of diet, we are now continuing drug treatment and two-bottle testing to see when Wnt inhibition begins to affect taste sensitivity to bitter.

Identification of Wnt/ β -catenin responsive subtypes of taste bud cells and investigating the phenotype of mouse models like the Dkk1-overexpressing mice are necessary to further understand the function of Wnt/ β -catenin in taste bud renewal.

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LOCALIZATION AND INTERACTIONS OF RNA BINDING PROTEINS VIA FLUORESCENCE MICROSCOPY AND FRET
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We are interested in understanding in greater detail the interactions of a class of RNA binding proteins, known as ARE binding proteins, that recognize and bind to A+U rich elements (AREs) within the 3'UTRs of target mRNA molecules. Members of this protein class can either stabilize (HuR), or destabilize (AUF1, KSRP) ARE containing RNAs, or, can hold them in a state of translational arrest (TIA-1). Studying the interactions of these ARE binding proteins will lead to more complete understanding how they regulate the turnover of the ARE containing RNAs.

In the current investigation, we used fluorescent microscopy and Fluorescent Resonance Energy Transfer (FRET) to study protein/protein interactions of endogenous and exogenous ARE binding proteins in live and fixed cells. Using FRET, we had previously demonstrated homo- and heteromeric interactions between two ARE mRNA binding proteins, AUF1 and HuR. Here, we expand on these findings to investigate heteromeric interactions between these and other ARE mRNA binding proteins (BP) and investigate their interactions within specific intracellular compartments, stress granules (SG) and P-bodies (PB), and under certain cellular conditions. In the resting state, all mRNA BP examined (endogenous and exogenous AUF1, HuR, KSRP and TIA-1) were readily detectable in the nucleus (N). In contrast, cytoplasmic (C) expression was variable with HuR and TIA-1 being relatively more prevalent. Stimulation of MAP Kinase activity, which has been shown to stabilize ARE-containing mRNAs, caused marked N/C shuttling of AUF1 and HuR but had no detectable effect on relocalization of TIA-1 or KSRP. Induction of oxidative stress with arsenite resulted in robust formation of stress granules with HuR and TIA-1 being readily detectable in SG and AUF1 and KSRP less abundant.

Using ImmunoFRET AUF1 was found to interact with HuR and KSRP, pair-wise, in N, C, and in SG, when formed. In marked contrast,

AUF1 and KSRP interacted with TIA-1 in N and SG only. Interestingly, we found that TIA-1 was the only ARE BP that shows a presence in PB. These results demonstrate that all the ARE BP studied, both stabilizing and destabilizing, interact.

In conclusion, this finding leads to the understanding that the regulation of ARE-containing mRNA molecules by ARE binding proteins is complex and involves a hierarchy of protein-protein and protein-RNA interactions. We conclude that the FRET and immuno-FRET are useful techniques capable of differentiating apparent co-localization of proteins from actual physical proximity/interaction.

INFECTION WITH GAMMAHERPESVIRUS 68 SUPPRESSES ANTIBODY RESPONSES.

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Acute infection with murine gammaherpesvirus 68 causes a transient inhibition of antibody responses initiated during infection. Both antibody responses to T cell dependent and T cell-independent antigens are suppressed and the antibody responses are suppressed for at least 1 month after infection. Adoptive transfer studies demonstrated that the environment in infected mice causes the suppression, as B cells from uninfected mice lose their ability to initiate an antibody response upon transfer into an infected mouse, whereas B cell from infected mice regain their ability to initiate an antibody response upon transfer into uninfected mice. B cells isolated from infected mice display some features associated with B cell anergy, such as surface IgM down regulation and dampened calcium mobilization upon IgM aggregation.

The results are discussed in light of the clinically observed immune unresponsiveness associated with the inflammatory responses during severe infections or trauma.

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THE IMPACT OF OBESITY ON ENERGY BALANCE, FUEL UTILIZATION AND PHYSICAL ACTIVITY DURING THE ESTROUS CYCLE AND FOLLOWING SURGICAL OVARIECTOMY IN RATS.

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Background: The impact of obesity on energy homeostasis throughout lifespan is critical to our understanding of how obesity affects disease. In the present study, energy balance and fuel utilization were followed in lean (L) and obese (OB) rats over the estrous cycle and during the period of rapid weight gain following surgical ovariectomy (OVX).

Methods: Female Wistar rats were fed a high fat (46%kcal) diet for 16 wks to produce mature OB and L rats (29±1 vs. 22±1 %fat). The stage of estrous was determined by daily vaginal lavage, while animals were examined in a comprehensive metabolic monitoring system. Metabolic measurements were repeated during the 3-wk period of rapid OVX-induced weight gain.

Results: Obese rats exhibited a larger fluctuation in energy balance across the cycle and were less active both before and after OVX ($p < 0.001$). While less active, OB rats expended more energy for each movement, such that activity thermogenesis and nonresting energy expenditure were similar for the two groups. Obese rats displayed a greater dependence on carbohydrate for energy over the cycle, and experienced a blunted energy imbalance during OVX-induced weight gain.

Conclusion: Obesity was accompanied by large fluctuations in energy balance across the cycle, less activity, a greater dependence on ingested carbohydrate, and an impaired response to OVX. These observations reveal important experimental considerations for studies of obesity and energy balance in female preclinical models. In addition, these effects of obesity on homeostatic regulation over the cycle and in response to the menopausal transition may have important health implications.

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ROLE OF PDZ PROTEINS IN THE DIFFERENTIAL REGULATION OF THE PROXIMAL TUBULE NAPI TRANSPORTERS.

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The Na-dependent phosphate (NaPi) transporters NaPi-2a and NaPi-2c play a major role in the renal reabsorption of Pi and maintenance of Pi homeostasis. The transporters show differential regulation under dietary and hormonal stimuli suggesting different regulatory pathways controlling the endocytosis, stability, or functionality of the transporters. The scaffolding NHERF family of PDZ proteins has been involved in the regulation of NaPi-2a and NaPi-2c. We propose that differences in the molecular interaction with these PDZ proteins are related with the differential adaptation of the transporters. We studied the specific interaction of NaPi-2a and NaPi-2c with NHERF-1 and -3 in the OKP cells by FLIM-FRET lifetime measurements. Results showed a direct interaction of NaPi-2a with NHERF-1 (FRET occurrence), while there was a much smaller interaction between NaPi-2c and NHERF-1. In contrast NaPi-2c showed significant FRET with NHERF-3 protein. According with these results, adaptation of the NHERF-3 KO mouse to chronic low Pi diets was impaired in the case of NaPi-2c but not in NaPi-2a. In response to a low Pi diet NHERF-3 KO mice showed robust upregulation of NaPi-2a but decreased adaptive response of NaPi-2c. These results indicate that differential affinity of the NaPi transporters for NHERF-1 and -3 proteins could partially explain their differential regulation. Interaction between NaPi-2c and NHERF-3 seems to play an important role in the physiological regulation of NaPi-2c.

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REGULATION OF VIRUS-SPECIFIC CD4⁺ T CELL FUNCTION BY MULTIPLE COSTIMULATORY RECEPTORS DURING CHRONIC HIV INFECTION

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Elevated expression of inhibitory receptors on virus-specific T cells has been implicated as a mechanism by which viruses evade host immune surveillance. Blockade of these pathways during chronic infection leads to increased T cell function and improved immune control of viral replication. To explore the association between costimulatory receptors and HIV replication, we examined the expression of PD-1, CTLA-4, TIM-3 and CD28 on HIV-specific CD4⁺ T cells from HIV infected subjects. Greater than 30% of HIV-specific CD4⁺ T cells from untreated subjects coexpressed PD-1, CTLA-4 and TIM-3, while <2% of CMV- or VZV-specific CD4⁺ T cells expressed all three receptors. Expression of these inhibitory receptors correlated with one another, with strongest correlation between PD-1 and CTLA-4. Coexpression of all three inhibitory receptors on HIV-specific CD4⁺ T cells was strongly correlated with viral load as compared with expression of each receptor individually. Suppression of HIV replication with antiretroviral therapy was associated with decreased expression of all three inhibitory receptors on HIV-specific CD4⁺ T cells. *In vitro* blockade of PD-1 binding concurrent with stimulation through CD28 synergistically increased HIV-specific CD4⁺ T cell proliferation to a greater extent than either alone, although enhanced proliferation was associated with increased expression of inhibitory receptors and CCR5. These findings indicate that HIV-specific CD4⁺ T cell responses during chronic infection are regulated by complex patterns of coexpressed inhibitory receptors and that the synergistic effect of inhibitory receptor blockade

and stimulation of costimulatory receptors could be used for therapeutic augmentation of HIV-specific CD4⁺ T cell function.

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THE ROLE OF ING4 AND ING5 TUMOR SUPPRESSORS IN CHROMATIN REMODELING AND DISEASE.

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The Inhibitor of Growth (ING) family of tumor suppressors has been implicated in diverse nuclear processes that regulate cellular viability and tumorigenesis. Various histone modifications are linked to discrete chromatin states, and regulate the extent of accessibility of DNA by transacting factors. One of the most common marks associated with euchromatic genomic regions is methylation of histone H3 at lysine 4 (H3K4). H3K4 can be mono-, di- or tri-methylated, with tri-methylation (H3K4me3) associated with active chromatin and gene transcription. The C-terminal PHD fingers of ING4 and ING5 bind these modified histone tails, and have been established as critical components of the Jade/HBO1 and MOZ/MORF histone acetyltransferase (HAT) complexes, respectively. However, the functional role of these PHD modules in the HAT complexes has not been determined.

In this study we aimed to elucidate the specificity determinants of the ING4 and ING5 PHD fingers towards histone tails using X-ray crystallography, NMR, fluorescence spectroscopy and mutagenesis. Here we report the crystal structures of ING4 and ING5 in complex with the H3K4me3 histone peptide at 1.8 Å and 1.75 Å resolution, respectively. The peptide binding affinities were measured using tryptophan fluorescence, and residues important for the histone binding interactions were identified by mutagenesis. Functional analysis of the MOZ/MORF and HBO1 HAT complexes indicates that both the native ING4/5

subunits and methylated H3K4 are necessary for their enzymatic activity. NMR chemical shift perturbation experiments reveal residues important for the binding interaction between the N-terminus of ING4/5 and the Jade1/BRPF1 subunits of the HAT complexes. Together these studies provide an essential link between HAT activity and chromatin remodeling, and impart a greater understanding on how the histone code is read.

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WP1066 SLOWS THE PROGRESSION OF PILOCARPINE-INDUCED EPILEPSY BY INHIBITING THE PHOSPHORYLATION OF STAT3

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Pilocarpine-induced status epilepticus (SE) activates the JAK/STAT pathway in the dentate gyrus (DG) of the hippocampus. Decreased transcription of GABA_A Receptor α 1 subunit (GABA_R α 1) after SE is mediated by ICER and phosphorylated cAMP response element-binding protein (CREB) binding to the GABA_R α 1 CRE site. Infusion of pyridone 6 (P6), a pan-JAK inhibitor, blocks JAK/STAT pathway activation after SE as shown by the absence of increases in pSTAT3 protein levels, blockage of increases in protein and mRNA expression of inducible cAMP early repressor (ICER), and the consequential reduction in downregulation of the α 1 subunit in the DG. These distinctive properties suggest that JAK/STAT inhibitors may potentially be a novel addition to current therapy for refractory seizures, which occur in ~40% of temporal lobe epilepsy (TLE) patients. Current studies demonstrate that spontaneous recurrent seizures that define chronic epilepsy "reactivate" the JAK/STAT pathway, specifically that increased levels of pSTAT3 are expressed in the DG within 3 hours of a spontaneous seizure in pilocarpine-treated rats 4 weeks after SE relative to control rats that received a subconvulsive dose of pilocarpine, did not experience SE, and did not have spontaneous seizures. Continuous video-EEG monitoring for two weeks demonstrated that administration of i.p. WP1066 at onset of pilocarpine-induced SE alters the progression of epileptogenesis. Latency to first seizure was not affected by WP1066 administration; and the first spontaneous seizure appeared at 3-7 days after SE for control rats and 5-8 days after SE for WP1066 treated rats. This initial data suggests that 100-150 mg/kg total WP1066 treatment at SE may slow the progression of increasing seizure frequencies over time inherent to post-SE models. Continuous video-EEG monitoring from SE for

a longer duration with more animals is required to further evaluate this hypothesis.

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INHIBITION OF AUTOPHAGY SUPPRESSES LYMPHOMA CELL KILLING BY FAS LIGAND

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The role of autophagy in cancer therapy is equivocal; there are substantial data indicating that autophagy protects cells from chemotherapeutics but there is also mounting evidence that chemotherapeutics may kill cells via an apoptosis-independent, cell death program which depends on autophagy. The mechanisms responsible for these disparate effects are unknown. In order to understand the mechanism behind these contradictory data I have taken advantage of the observation that lymphoma cell killing mediated by Fas ligand, a potent inducer of extrinsic apoptosis, is suppressed by autophagy inhibition. This autophagy-dependent effect on cell killing is not seen with the closely related death receptor ligand TRAIL, which activates apoptosis by a nearly identical pathway. I am working to understand at a cellular and molecular level, how inhibition of autophagy may suppress cell death (apoptosis, autophagic cell death or necrosis). This work will enable us to better understand the differences between these two similar cell death pathways and how autophagy manipulation might be beneficial, or even detrimental, in the treatment of lymphoma via death receptor agonists.

MOLECULAR DETERMINANTS OF RECEPTOR SPECIFICITY AND HOST RANGE OF SPIKE PROTEINS OF GROUP 1 CORONAVIRUSES

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Coronaviruses (CoVs) in group 1 utilize several different receptor proteins. NL63, like SARS-CoV (a group 2a virus), uses human angiotensin-converting enzyme 2 (hACE2) as its receptor. Structural studies have shown that 3 receptor binding motif (RBM) loops in the S1 domain of NL63 spike bind to a site on hACE2 that is also recognized by SARS-CoV spike protein. Other group 1 CoVs, including feline and canine coronaviruses, transmissible gastroenteritis virus of swine and human coronavirus 229E use aminopeptidase N (APN) as their receptors. Loops structurally similar to the RBMs of NL63 spike protein were predicted in the spike proteins of HCoV 229E and feline coronavirus FIPV. To determine if these regions function as RBMs for APN, we constructed chimeric FIPV receptor binding domains (RBDs) that contain loops 1, 2, or 3 of NL63 spike in place of the predicted FIPV loops. We tested the binding of the chimeric and native RBDs to cells expressing feline APN by flow cytometry, and used ELISA to test binding of wild type or chimeric RBDs to soluble feline APN. Interestingly, 2 of the 3 predicted loops in FIPV RBD are required for binding specifically to feline APN. The co-crystal of the RBD of NL63 bound to its hACE2 receptor showed that 3 RBM loops bind to the receptor (Wu et al., PNAS 106:19970, 2009). We showed that 3 predicted loops of spike proteins of group 1a CoVs also are critical for binding to the APN receptor glycoprotein. Thus for two distantly related CoVs in group 1, the receptor-binding domains are likely structurally similar, with a core and 3 projecting loops. Amino acid changes in these key receptor-binding loops may account for differences in group 1 coronavirus host range, receptor specificity and evolution.

CHOLINERGIC MODULATION OF EVOKED EXCITATORY CURRENTS AT THE MOSSY FIBER-CA3 SYNAPSE IN THE MAMMALIAN HIPPOCAMPUS.

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Nicotine, from tobacco smoke exerts its effects on the brain by activating nicotinic acetylcholine receptors (nAChRs). We have previously shown that activation of these receptors by nicotine results in a burst of spontaneous glutamate release at the mossy fiber terminals of the hippocampus. This nAChR-mediated release can drive the postsynaptic neuron above its firing threshold in what represents a unique presynaptic action potential-independent form of synaptic transmission. Our results suggest that nicotine could potentially hijack this synapse by strengthening it in a manner independent of a physiological context, but dependent on the presence of the drug.

In this study, we ask how action potential-independent actions of nicotine affect synaptic glutamatergic responses mediated by stimulation of the presynaptic neuron (eEPSCs).

Hippocampal slices were prepared from 2-3 week old FVB mice. Whole-cell voltage-clamp recording was performed at holding potential of -60 mV from pyramidal cells in the CA3 region. Agonist (1 mM Acetylcholine and 1 μ M Atropine; ACh/At) was pressure applied using a picospritzer and puffer pipettes positioned at the stratum lucidum, ~20 μ m away the cell being recorded from. A train of five pulses at 50 Hz was given every 10 sec and eEPSCs were evoked using a glass stimulating electrode placed at the dentate gyrus. The degree of facilitation and peak amplitude were calculated by averaging the size of control eEPSCs measured before the ACh/At application and comparing it to the average size of eEPSCs in the selected time windows (during bursting sEPSCs, 1 min wash-out and 5 min wash-out) after agonist application. MF-eEPSC amplitudes were significantly facilitated by the pulse trains ($P < 0.05$). The amplitude of the fifth peak was not changed during the bursting period and was maintained during wash-out period. But the first peak was decreased after a 1 min wash-out period and then recovered to

control levels. These result shows that the nAChR-induced burst of spontaneous glutamate release reduces unitary eEPSCs possibly by altering the readily releasable pool at this synapse.

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NEURAL RYR1 MEDIATES VESICLE RELEASE, MOTONEURON ENDPLATE DISTRIBUTION AND RELEASE OF AGRIN

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Proper motor axon connectivity and activation of the neuromuscular junction (NMJ) are required for locomotion and respiration. Defects in synaptic transmission and release can lead to neuromuscular disorders in humans. To provide direct insight into these fundamental processes, a forward genetic screen in mice was performed to identify recessive mutations that disrupt locomotor development by scoring for defects in reflex movements. The mutagenized mouse model *line-1-4* was identified with specific deficits in locomotion and a non-motility phenotype throughout the body. Motor axons grow past pre-patterned NMJs and centralized motor endplate regions are decreased in diameter, while in few instances axon fail to innervate the diaphragm. Moreover, the identified missense mutation was in the Ryanodine receptor type 1 (*Ryr1^{LN1}*), a receptor considered to be more skeletal than presynaptic. Interestingly, sharp electrode recordings of *Ryr1^{LN1}* muscle show a loss of evoked transmission that is rescued by addition of Ryr agonists, increased extracellular calcium, and thapsigargin, suggesting a presynaptic defect in calcium induced calcium release (CICR). This rescue of presynaptic function was extended into a novel in vitro hindbrain-diaphragm preparations that show new synapses form adjacent to overextended *Ryr1^{LN1}* mutant motor axons. Moreover, this new synapse formation is inhibited through the application of antibodies to Agrin. Thus, activation of the RyR1 receptor may be responsible for the activity dependent release of agrin at levels that would properly distribute the endplates along defasciculated axons. These data suggest a role for *Ryr1^{LN1}* during activity-dependent synaptic transmission on the pre-synaptic side the NMJ. In *Ryr1^{LN1}* mutant diaphragms that lack motor axons, pre-patterned NMJ's form. However, instead of a centralized endplate region, NMJs were spread throughout the diaphragm muscle suggesting a

novel role for Ryr1 on the post-synaptic side of the NMJ.

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MOLECULAR BASIS OF MEMBRANE TARGETING BY THE PLECKSTRIN HOMOLOGY DOMAIN OF GENERAL RECEPTOR FOR PHOSPHOINOSITIDES 1

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Pleckstrin homology (PH) domains regulate a wide range of biological processes including cell proliferation and survival, signal transduction, membrane trafficking and cytoskeletal rearrangement through binding to phosphoinositide lipids. The PH domain of GRP1 (general receptor for phosphoinositides) binds PI(3,4,5)P3 with high affinity and specificity, however the overall molecular mechanism of membrane recruitment and docking by GRP1 remains unclear. Here we show that the membrane anchoring of the GRP1 PH domain involves the specific recognition of PI(3,4,5)P3, which is facilitated by non-specific electrostatic interactions with acidic lipids and accompanied by a hydrophobic insertion into the bilayer. Our data indicate that the association of the GRP1 PH domain with membranes is regulated by the acidic cellular environment. The membrane insertion interfaces, binding parameters and regulation of the PI(3,4,5)P3 binding by pH are investigated by NMR, liposome binding, monolayer surface tension, and mutagenesis experiments. The functional significance of the pH sensitivity and the effect of acidic and basic media on the phosphoinositide recognition, hydrophobic insertion and non-specific electrostatic contacts are established.

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DECLINING LYMPHOID PROGENITOR FITNESS PROMOTES AGING-ASSOCIATED LEUKEMOGENESIS

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Aging is associated with the functional decline of cells, tissues, and organs. In addition, age is the single most important prognostic factor in the development of most human cancers, including chronic myelogenous and acute lymphoblastic leukemias initiated by Bcr-Abl oncogenic translocations. Prevailing paradigms attribute the association between aging and cancers to the accumulation of oncogenic mutations over time, as the incidence of oncogenic events is thought to limit initiation and progression of cancers.

On the other hand, aging-associated functional decline (caused by both cell autonomous and non-cell autonomous mechanisms) is likely to reduce the fitness of stem/progenitor cell populations. This reduction in fitness should be conducive for increased selection of oncogenic mutations which may at least partially alleviate fitness defects, thereby promoting the initiation of cancers. We tested this hypothesis using mouse hematopoietic models. Our studies demonstrate that the dramatic decline in the fitness of aged B-lymphopoiesis coincides with global alterations in gene expression, inappropriate expression of lineage specific genes, and reduced receptor-associated kinase signaling (Akt and STAT5). We further show that Bcr-Abl provides a much greater competitive advantage to old B-

lymphoid progenitors compared to young B-lymphoid progenitors, coinciding with restored kinase signaling pathways. This enhanced competitive advantage translates into increased promotion of Bcr-Abl driven leukemias which is more apparent in an aged background. These studies support an unappreciated causative link between aging and cancer: increased selection of oncogenic mutations as a result of age-dependent alterations of the fitness landscape.

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IDENTIFICATION AND CHARACTERIZATION OF *RAD53-REP*, A NOVEL SEPARATION OF FUNCTION ALLELE DEFECTIVE IN DNA REPLICATION.

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The protein kinase Rad53/Chk2 is central to activation of DNA damage and replication checkpoints, and it plays an essential role in upregulating nucleotide levels. Genetic studies in budding yeast suggests Rad53 plays a novel role in controlling initiation of DNA replication, and this function is independent of Rad53-mediated regulation of the DNA damage checkpoint and of nucleotide levels. The *rad53Δ* allele is synthetically lethal with certain alleles of *CDC7*, a protein kinase that is essential for initiation of DNA replication. Rad53 is also required for *mcm5-bob1*-mediated bypass of *CDC7*. Mutations of checkpoint genes that function upstream or downstream of Rad53 or in the mitotic checkpoint do not display this genetic interaction with *CDC7*. We have isolated an allele of *RAD53*, *rad53-rep*, that is checkpoint proficient but is synthetically lethal with *cdc7-1*, thus providing further evidence that the checkpoint function of *RAD53* is functionally separable from the DNA replication function. *rad53-rep* contains six mutations in its kinase domain, but a *rad53-rep* mutant can autophosphorylate Rad53p in the DNA replication checkpoint response. Wild type Rad53 physically interacts with origins of replication in the one hybrid assay, and this interaction is mediated through the Rad53 kinase domain. *Rad53-rep* p is defective in interacting with origin DNA in the one hybrid assay, suggesting interaction of Rad53 with origins of replication may be related with Rad53's role in DNA replication. Both *rad53Δ* and *rad53-rep* mutants display a chromosome loss phenotype, providing further evidence that Rad53 plays a role in the initiation of DNA replication. Deletion of the major H3/H4 gene pair partially suppresses specific growth defects in *rad53Δ* mutants. Deletion of the major histone H3/H4 pair suppresses both *rad53Δ-cdc7-1* and *rad53-rep-cdc7-1* synthetic lethality, and both *rad53Δ* and *rad53-rep* mutants are sensitive to overexpression of the major H3/H4

gene pair. These findings suggest Rad53 may regulate DNA replication initiation by altering chromatin structure at origins of DNA replication. Identification and characterization of *rad53-rep* provides further evidence that Rad53 functions in DNA replication initiation independently of its role in the DNA damage checkpoints and that the role of Rad53 in DNA replication is closely related to chromatin configuration.

ROLE OF SIX1 IN MAMMARY TUMOR INITIATING CELLS.

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The homeobox transcription factor Six1 plays an important role in normal embryonic development. We recently demonstrated that ectopic expression of Six1 in the mammary gland of mice induced tumor formation with activated Wnt signaling and epithelial-mesenchymal transition (EMT), characteristics of cancer stem/progenitor cells. In this study, we examined whether Six1 is involved in expansion or maintenance of normal mammary stem cells (MSC) and mammary tumor initiating cells (TIC).

To determine whether Six1 modulates the MSCs, we ectopically expressed Six1 in the mouse mammary gland, and examined the MSCs using both flow cytometry and mammosphere assays. We found that Six1 overexpressing animals had twice as many CD24⁺/CD29^{hi} MSCs than control animals. In the mammosphere assay, which measures the functional MSCs, we further showed that Six1 expressing mouse cells had more than a 20-fold increase in mammosphere formation. These results demonstrate that Six1 overexpression regulates MSCs expansion and/or maintenance.

Because ectopic, mammary-specific Six1 expression in transgenic mice induces mammary tumors with EMT and stem cell features, and because both EMT and stem cell characteristics are associated with metastasis, we developed a second model to specifically analyze the role of Six1 in metastasis. To develop this model, we took the poorly metastatic MCF7 cells and overexpressed Six1. MCF7-Six1 induced EMT both in vitro and in vivo, and also induced metastasis when orthotopically injected into nude mice. Gene expression profiles demonstrated that MCF7-Six1 displayed a characteristic stem cell signature. In addition, the CD44⁺/CD24^{lo} human mammary TIC population was increased 5 fold in MCF7-Six1 as compared to control cells. Finally, functional TICs were also increased by Six1 overexpression, as shown by the 2-fold

increase in mammosphere formation, and as suggested by orthotopic transplantation studies in NOD/SCID mice.

We have previously shown that Six1 induces EMT and metastasis by increasing TGF- β signaling. Because EMT and metastasis are linked to stem cells, we analyzed whether TGF- β is important for the Six1-induced increase in TICs. Inhibition of TGF- β signaling decreased the TIC population in MCF7-Six1, as measured by flow cytometry and mammosphere assays. Fine tuning of the pathway showed that TGF- β induced ERK phosphorylation appears important for the increased TICs in response to Six1 overexpression, since inhibition of MEK1/2 dramatically decreased the TIC population in MCF7-Six1. Together, these results demonstrate that Six1 plays a role in the expansion or maintenance of the MSC and TIC population, through its ability to activate ERK in a TGF- β dependent manner.

IDENTIFICATION AND CHARACTERIZATION OF A LOSS-OF-FUNCTION HUMAN *MPYS* VARIANT.

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MPYS, also known as STING and MITA, is a Type I IFN stimulator essential for host defense against RNA, DNA viruses and intracellular bacteria. Here we report identification of a loss-of-function human *MPYS* haplotype that contains three non-synonymous SNPs, R71H-G230A-R293Q (thus named the *HAQ* haplotype). We found ~3% of Americans are homozygous for this *HAQ* haplotype. Importantly, cells expressing *HAQ* have dramatically decreased Type I IFN production upon *Listeria monocytogenes* infection. Efforts to understand the mechanism of this defect led to the discoveries that MPYS possesses reductase activity. This activity is required for Type I IFN stimulation and lost in *HAQ* MPYS. Finally, we show endogenous reactive oxygen species inhibits MPYS mediated *Listeria monocytogenes* induced Type I IFN production in macrophages by inhibiting its reductase activity. These studies provide a mechanistic link between oxidative stress and innate immunity and suggest that Individuals carrying *HAQ* may exhibit heightened susceptibility to viral infection but enhanced resistance to intracellular bacteria infection.

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FUNCTIONAL CONSEQUENCES OF COUPLING 3'-END PROCESSING TO mRNA EXPORT VIA THE PCF11-YRA1 INTERACTION

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In eukaryotes ribonucleoprotein particle (mRNP) assembly is a complex, multifaceted process that involves many integrated steps. Transcription by RNA polymerase II (pol II) is coordinated with mRNA processing events, e.g. capping, splicing, cleavage, and polyadenylation, as well as with the loading (and unloading) of mRNA binding proteins. Such coordination ensures the accuracy of each step and promotes efficient packaging of the nascent mRNA into an export-competent mRNP.

Co-transcriptional loading of mRNA binding proteins, particularly export adaptor proteins, is an important feature of normal mRNP biogenesis. In yeast, export adaptor proteins such as Yra1 couple the mRNP to export receptors, which in turn bind the nuclear pore. Recently we discovered that Yra1 recruitment to actively transcribed genes requires the 3'-end processing machinery. Specifically, Yra1 recruitment to elongating pol II is mediated by a direct physical interaction between the Pcf11 subunit of cleavage factor IA (CFIA) and Yra1.

CFIA consists of four subunits (Rna14, Rna15, Pcf11 and Clp1) and is recruited progressively within the coding regions of pol II transcribed genes. Importantly, this recruitment depends upon direct binding of Pcf11 to pol II. The Pcf11-pol II interaction acts to couple transcription elongation to 3'-end formation and transcription termination. Interestingly because Pcf11 binds pol II and is a subunit of CFIA, the Pcf11-Yra1 interaction places Yra1 in a position to potentially influence both transcription and 3'-end processing.

Here we utilize RNase protection assays (RPA) to demonstrate that loss of Yra1 protein disrupts normal poly(A) site choice/cleavage on the *ACT1* gene *in vivo*, shifting cleavage towards ORF proximal poly(A) sites. This "upstream" shift may reflect faster kinetics of the cleavage reaction, and suggests that wild type Yra1 negatively regulates Pcf11/CFIA activity. This supposition is further supported by

data showing that 1) Pcf11 disengages from Yra1 at or near the poly(A) site(s) of many genes (determined by ChIP-chip), and 2) Yra1 can prevent full assembly of CFIA by excluding the Clp1 subunit (determined by *in vitro* competition assays).

Collectively these results suggest that the Pcf11-Yra1 interaction may contribute to the formation of a poised, but inactive form of CFIA that lacks Clp1. In this model, the exchange of Yra1 for Clp1 at the 3'-ends of genes would complete and activate CFIA, allowing cleavage/polyadenylation to occur and the deposition of Yra1 onto the mRNA. Additional studies to determine whether the Pcf11-Yra1 interaction influences pol II transcription are underway.

PLASMA METABOLITES IN THE MAMMALIAN HIBERNATION CYCLE

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Hibernation is a dynamic endogenous circannual rhythm in which metabolism, heart rate and body temperature all decrease drastically through most of the winter season in order to conserve energy. Hibernators periodically and regularly rewarm, and these intermittent euthermic periods are referred to as interbout arousals. Although the purpose behind the interbout arousal is not yet known, it is hypothesized that they are essential for maintaining biochemical homeostasis. We hypothesize that these physiological changes are reflected in biochemical changes that provide mechanistic insights into, and biomarkers for, hibernation states. In this study, we sought to identify compounds that are significantly different in the plasma of a hibernator, the thirteen-lined ground squirrel (*Ictidomys tridecemlineatus*), throughout the seasons of the year. Quantities of more than 200 metabolites were determined using LC and GC mass spectrometry, and quantitative differences in compounds among the seasonal groups were determined by statistical analyses and several machine learning classification tools. Twenty compounds were identified that distinguish plasma among the eight different stages of hibernation. Our findings using machine learning tools such as random forests are consistent with a proposed two-switch model of hibernation in which setting the summer-winter switch to winter enables expression of a distinct winter torpor-arousal switch.

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ADENOSINE A2B RECEPTOR SIGNALING ATTENUATES ACUTE LUNG INJURY

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Acute lung injury (ALI) is among the leading causes of morbidity and mortality of critical illness. Functional studies in gene-targeted mice for the A2B receptor indicate a more severe course of disease. The A2B adenosine receptor (AR) is known to possess a hypoxia responsive element (HRE) which is bound by hypoxia-inducible factor (HIF-1 α) and has been implicated in increased signaling under conditions of stress and inflammation, along with upregulated levels of adenosine. We wanted to see whether the same regulation occurred under conditions of mechanical stretch *in vitro* and *in vivo*. Initial insight was gained from studies of cultured endothelia and epithelia exposed to cyclic mechanical stretch, showing a time-dependent induction of the A2BAR. Furthermore, constructs containing a truncated and subsequently mutated HRE showed dysfunctional A2BAR expression under mechanical stretch. CHIP analysis revealed the role of HIF-1 α in the transcriptional upregulation of the A2BAR under these conditions. Functional studies of ventilator-induced lung injury (VILI) indicated that pharmacological inhibition of the HIF protein was associated with decreased levels of A2BAR receptor expression, alongside dramatic increases in lung inflammation. These studies support the role of HIF-1 in regulating the A2BAR under mechanical stretch, and implicate a role for it in coordinating the pathological effects of the A2BAR in ALI, identifying it once again as a potential therapeutic target.

TECHNIQUES TO IMPROVE THE EFFICIENCY OF A MIDDLE EAR IMPLANT: EFFECT OF DIFFERENT COUPLING METHODS TO THE OSSICULAR CHAIN.

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Active middle ear implants (AMEIs) provide advantages over conventional hearing aids in the rehabilitation of sensorineural hearing loss. Efficient transfer of energy from the AMEI to ossicular chain (OC) is desired to maximize output and optimize gain. The aim of this study was to objectively assess the performance of an AMEI using five different methods of coupling to the OC. Six temporal bones were prepared to expose the OC. The Otologics Gen II MET-V™ was coupled to the OC via: 1) direct contact on the body of the incus (baseline); 2) insertion of transducer tip into a laser-generated hole in the body of the incus; 3) an àWengen clip to the long process of the incus; 4) a 0.5 mm diameter cylinder to the long process of the incus; and 5) a bell-shaped prosthesis in contact with the stapes. The performance of the AMEI was assessed by stapes velocities (H_{EV}) and the maximum equivalent ear canal sound pressure levels ($L_{E_{max}}$) were computed. The H_{TV} before and after loading the transducer was measured to determine the loading effect. Data were analyzed in three frequency ranges: low (0.25-1 kHz); medium (1-3 kHz) and high (3-8 kHz). The AMEI in contact with the incus without a laser hole produced $L_{E_{max}}$ of 112, 126 and 122 dB in low, medium and high frequency ranges, respectively, while the change in H_{EV} was -4, -2 and 2 dB. Seating the tip in a laser hole significantly improved $L_{E_{max}}$ by 6 dB only at high frequencies. Using the àWengen clip significantly improved $L_{E_{max}}$ by 14, 11 and 19 dB by frequency range compared to baseline

without negative loading effects. Cylinder tip coupling improved $L_{E_{max}}$ by 9, 10 and 11 dB in the respective frequency ranges, without negative loading effects. Coupling via the bell-shaped tip improved $L_{E_{max}}$ by 22, 17 and 16 dB in the respective frequency ranges ($p < 0.0001$). Stapes velocity generated by the AMEI transducer is influenced substantially by the coupling technique to the OC and placement of the stimulating tip along the incus.

This study was supported by an Otologics LLC educational grant and by Dept of Physiology and Biophysics funds.

P38 MITOGEN-ACTIVATED PROTEIN KINASE-DRIVEN MAPKAPK2 REGULATES INVASION OF BLADDER CANCER BY MODULATION OF MMP-2 AND MMP-9 ACTIVITY

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Transitional cell carcinoma (TCC) is the most common form of bladder cancer. Though MMP-2 and MMP-9 have been shown to play an important role in bladder cancer metastasis, the molecular mechanisms that regulate metastasis in bladder cancer are not completely understood. Over expression of these matrixes metalloproteinase correlated significantly to disease-specific survival, and showed an independent prognostic value as a biomarker in bladder cancer. The present study was aimed at determining the role of p38 mitogen activated protein kinase (MAPK) driven mitogen activated protein kinase-activated protein kinase 2 (MAPKAPK2) in regulation and modulation of bladder cancer metastasis. We used two bladder cancer cells lines, HTB9 and HTB5 derived from different tumor stages. Using multiple approaches, our results suggest that p38 MAPK modulates MMP-2/9 activity, expression and invasion of bladder cancer cells. Additionally, p38MAPK regulates MMP-2 at transcriptional level by stabilizing MMP-2 and MMP-9 mRNA transcript. Furthermore, we found that MAPKAPK2 is a downstream effector of p38 MAPK signaling pathways, and were associated with MMP-2/9 activity and therefore invasion of bladder cancer cells. Transient transfection of wild type and constitutive active MAPKAPK2 plasmid increases both MMP-2/9 activity and invasion, which were inhibited by suppression of p38 MAPK. Taken together, these studies demonstrate for the first time that bladder cancer invasion is orchestrated by p38 MAPK, and MAPKAPK2 is a down effector molecule

which in turn regulates invasion of bladder cancer by modulation of MMP-2 and MMP-9 mRNA stability.

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MER AND AXL RECEPTOR TYROSINE KINASES ARE NOVEL THERAPEUTIC TARGETS IN NON-SMALL CELL LUNG CANCER.

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Lung cancer is the primary cause of cancer mortality worldwide. Less than 25% of patients treated on current chemotherapy protocols survive 2 years, and new treatment strategies are needed to obtain improved patient outcomes. The identification of new targeted therapies which synergistically interact with chemotherapy is one approach to enhance patient survival. Axl and Mer are related receptor tyrosine kinases (RTKs) and are novel candidates for targeted inhibition since they are abnormally expressed and activated in many human cancers. In patient samples of non-small cell lung cancer (NSCLC), Axl overexpression has been statistically associated with metastatic disease. In this study, we used immunohistochemical staining to demonstrate that both Axl and Mer are expressed in NSCLC patient samples. In human NSCLC cell lines, qRT-PCR and immunoblotting indicate that Axl and Mer, as well as their ligands, are co-expressed suggesting that this family of RTKs may constitute an autocrine loop resulting in constitutive activity of these kinases. Knockdown of Axl or Mer expression in human NSCLC cell lines was accomplished via constitutive and inducible lentiviral short hairpin RNA constructs. In vitro assays of cell proliferation and survival indicated that constitutive knockdown of Axl or Mer did not have a significant impact on short-term growth of NSCLC cells. However, both Mer and Axl knockdown cells exhibited reduced long-term growth rates relative to control cells. To evaluate the role of Axl and Mer in apoptosis, we performed flow cytometric analysis of cells stained with YO-PRO-1 and propidium iodide. These experiments revealed that constitutive inhibition of Mer, but not Axl, results in increased induction of cell death suggesting that Axl and Mer may be contributing to NSCLC

proliferation and survival via distinct mechanisms. Additional in vitro assays demonstrated that inhibition of Mer or Axl significantly increased the sensitivity of NSCLC cells to numerous chemotherapeutic agents. Taken together, these results suggest that inhibition of Axl and/or Mer may increase the efficacy of chemotherapy and thereby improve patient outcome. While previous studies have suggested a role for Axl in the progression of NSCLC, these studies are the first to describe a role for the related receptor tyrosine kinase Mer in NSCLC or to define a potential synergistic role of Axl or Mer inhibition with commonly used chemotherapy agents. Our findings demonstrate that Mer and Axl are novel biological targets for treatment of NSCLC and possibly a spectrum of other cancers known to aberrantly express Mer and/or Axl.

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LEVERAGING EXISTING KNOWLEDGE TO IMPROVE BIOMEDICAL LANGUAGE UNDERSTANDING

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Large-scale language understanding of biomedical text is necessary in order to produce and extend the knowledge bases required to support next generation systems for genome-scale analysis of systems biology data. In addition to supporting the biologist, background knowledge plays an important role in language understanding. Most language understanding work is unidirectional in interacting with underlying knowledge: information is extracted from text and then stored in a knowledge base. However it is possible to leverage existing knowledge at language understanding time. Direct Memory Access Parsing (DMAP) is an approach to NLP that integrates its representations of text incrementally with existing knowledge as it reads; this is in contrast to systems that save memory integration for a final step.

This work presents an implementation of DMAP, OpenDMAP, an architecture designed to explore and evaluate the role existing knowledge can play in assisting the language understanding process. This allows experiments that have, for example, evaluated the role Gene Ontology (GO) annotations associated with specific proteins can play in recognizing and understanding “activation” events (specifically receptor and enzyme activation) in GeneRIFs. Leveraging this background knowledge, with minimal additional changes to the system, improved precision by 20%, while only reducing recall by 6%.

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B CELL LINEAGE PROGRESSION AND IDENTITY ARE IMPEDED SYNERGISTICALLY BY EBF1 AND RUNX1 HAPLOINSUFFICIENCY.

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The transcription factors Early B cell factor 1 (EBF1) and Runx1 are essential for proper B cell development. Previously, we demonstrated synergistic activity of EBF1 and Runx1 in the activation of the B cell-specific gene *mb-1* (*Cd79a*). *In vivo* and *in vitro*, B lymphopoiesis is arrested at an early state in the absence of EBF1. Hematopoiesis is ablated completely in Runx1-deficient fetuses, resulting embryonic lethality by day E14. To identify cooperative functions of these factors *in vivo*, we generated *Ebf1*(*E^{het}*)*Runx1*(*R^{het}*)-double haploinsufficient(*ER^{het}*) mice. Substantial compound effects were observed in *ER^{het}* mice. B cell numbers in the bone marrow and spleen were reduced dramatically. Generation of pre-B and immature B cells in *ER^{het}* mice was impeded by: 1) reductions in the frequencies of Ig light chain gene rearrangements and 2) significant alterations in the expression of B cell-specific genes. Notably, repression of c-kit and activation of Aiolos were delayed. Interestingly, a high percentage of pro- and pre-B cells from *ER^{het}* mice expressed the NK cell markers NK1.1, CD160 and CD244. Co-expression of these NK cell markers with genes indicative of B cell commitment was confirmed using multiplex, single cell PCR. Retroviral complementation of *Ebf1* and *Runx1* deficiencies eliminated the promiscuous expression of NK cell genes in *ER^{het}* bone marrow-derived pro-B cells. Moreover, activation of EBF1:ER with 4-OHT reduced the levels of NK cell-specific transcripts in EBF1-deficient fetal liver cells. Thus, appropriate dosage of *Ebf1* and *Runx1* is necessary for efficient activation of the B cell-specific program

and suppression of lineage inappropriate genes. Therefore, our data solidify EBF1's role in controlling B cell lineage commitment.

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POST-PARTUM MAMMARY GLAND INVOLUTION DRIVES BREAST CANCER PROGRESSION THROUGH COLLAGEN AND CYCLOOXYGENASE-2 AND IS INHIBITED BY NONSTEROIDAL ANTI-INFLAMMATORY DRUGS

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Women diagnosed with breast cancer within five years of a completed pregnancy are almost twice as likely to die from metastasis when compared to women who have not recently given birth. We define these cases as pregnancy associated, or PABC. Understanding of this disease has been severely limited by lack of pre-clinical models. We describe a mouse xenograft model of PABC that isolates the post-partum mammary gland involution window as a driving force for progression and metastasis of DCIS. In this model, DCIS cells exposed to the involuting mammary microenvironment (PABC) form larger, more numerous, proliferative tumors that are locally and systemically invasive, and able to seed the lung. We find the involution environment is characterized by high levels of radiating collagen fibers organized similar to collagen associated with invasive tumors. PABC tumors have increased levels of COX-2, as measured by IHC, and primary tumor cells isolated from PABC tumors are more motile in scratch and transwell filter assays in a COX-2 dependent manner. Evidence that COX-2 induced motility requires collagen signaling is demonstrated with functional blocking antibodies against $\beta 1$ -integrin, $\alpha 2\beta 1$ -integrin, and integrin associated protein CD47. We propose a mechanism by which post-partum involution collagen signaling through its receptor, $\alpha 2\beta 1$, upregulates COX-2 expression, which in turn increases migration of tumor cells. Utilizing this PABC model we demonstrate that treatment with the general COX1/2 inhibitor ibuprofen, restricted to involution, reduced tumor size, COX-2 expression and lung cell seeding, suggesting

that treatment of young mothers during post-partum involution may reduce metastasis of PABC.

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NUCLEOPROTEIN SPECIFIC CD8 T CELLS GENERATED BY ALUMINUM SALTS AND MONOPHOSPHORYL LIPID A PROTECT MICE FROM INFLUENZA INFECTION

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Influenza virus poses a major health and economic threat to the world's population. Current vaccines induce protective antibody responses to viral surface proteins. However, these proteins alter over time and a new vaccine must be developed annually. CD8 T cells, that can directly kill infected cells, recognize parts of the virus that are much less variable. Therefore, a vaccine that generates memory CD8 T cells might protect against annual variants and new emerging pandemic viral subtypes. Such a vaccine will require the addition of a safe but effective adjuvant that activates the innate compartment of the immune system, which is essential for an optimal T cell response. We have investigated two adjuvants that are currently licensed for use in humans in the USA, aluminum salts (alum) and the less toxic derivative of LPS, monophosphoryl lipid A (MPL). We have immunized mice with an influenza peptide conjugated to a carrier protein so that the only influenza specific response generated is to a single CD8 T cell epitope and have delivered this protein with either alum or MPL or a combination of both adjuvants. We examined the ability of T cells generated by these immunization protocols to protect mice from the morbidity caused by influenza A infection. Mice vaccinated with the peptide-protein conjugate

and either aluminum salts or MPL were partially protected. Immunization with the peptide-protein conjugate and both adjuvants provided enhanced protection. The combination of both adjuvants did not increase the number of T cells primed but did enhance their ability to kill target cells in vivo. This, and the finding that perforin knockout mice, in which T cells are unable to kill infected cells, were not protected, suggests that protection is mediated by cytotoxic T cells. These findings present a potential solution to the current problems of the influenza vaccine by providing a practical vaccine that uses adjuvants that are already approved for use in humans and that will offer protection to many viral subtypes.

The work was supported by HHMI, the NIH and the US Department of Defense.

INVESTIGATION OF THE INTERACTION BETWEEN MITOCHONDRIAL TRANSCRIPTION FACTOR A AND AMYLOID BETA AND IMPLICATIONS FOR ALZHEIMER'S DISEASE

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Mutations in the mitochondrial genome and malfunctions in mitochondrial transcription have been linked to numerous diseases such as muscular dystrophy, late onset Alzheimer's, type II diabetes, cancer, and cardiovascular disease. The proteins responsible for executing transcription include mitochondrial transcription factors A (TFAM), B1, and B2 and a mitochondrial RNA polymerase. In fact, an S12T mutation in TFAM has been identified in patients with late onset Alzheimer's, and the nuclear gene encoding for TFAM is thought to be involved with Alzheimer's according to alzegen.org. High levels of the amyloid beta proteins 1-40 and 1-42 (A β 1-40, and A β 1-42, respectively) have long been identified in extracellular plaques in the brains of patients with Alzheimer's, but these peptides are not present in the brains of healthy individuals. It has been shown that A β peptides can enter the mitochondria through the trans outer membrane transporter. Once inside the mitochondria, A β peptides are toxic to the mitochondria, cause a decrease in the expression levels of proteins encoded by the mitochondrial genome, and induce oxidative damage to mitochondrial DNA. In vivo over expression of TFAM attenuates this effect. We hypothesize that A β directly interacts with TFAM, and that this interaction inhibits proper mitochondrial DNA transcription and TFAM's ability to protect mitochondrial DNA from oxidative damage. In this study, we demonstrate (using isothermal titration calorimetry experiments) that TFAM does indeed bind A β 1-40 and A β 1-42, and that this interaction occurs at the C-terminal tail of TFAM. We also show that the TFAM/A β interaction inhibits TFAM's ability to bind and bend mitochondrial DNA using fluorescence resonance energy transfer assays. We are currently investigating A β 's ability to inhibit the production of mitochondrial RNA transcripts using *in vitro* mitochondrial transcription assays,

and we have started probing the nature of the A β /TFAM interaction at the molecular level using x-ray crystallography. Studies investigating the interaction between mutant TFAM S12T found in patients with late onset Alzheimer's and A β have recently been initiated. The TFAM/A β interaction likely occurs early in the progression of Alzheimer's disease, therefore, characterization of this interaction could lead to the development of novel treatments for Alzheimer's disease.

This work was funded by the Muscular Dystrophy Association (MDA).

INDIVIDUAL DIFFERENCES IN COCAINE RESPONSIVENESS AS A PREDICTOR OF ACQUISITION OF COCAINE SELF-ADMINISTRATION: A DOSE-RESPONSE STUDY

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Cocaine addiction remains a significant public health problem. Interestingly, it is estimated only 10 – 15% of individuals who try intranasal cocaine will progress to cocaine addiction (Gawin, 1991). Further, individual differences in the subjective effects of cocaine have been found to correlate with long-term cocaine use and dependence (Lambert et al., 2006). Therefore, understanding individual differences in cocaine responsiveness is critical for developing effective cocaine addiction prevention and treatment strategies. Similar to humans, individual differences in cocaine responsiveness are also seen in rats. Adult outbred Sprague-Dawley rats can be classified as either low or high cocaine responders (LCRs or HCRs) based on their locomotor response to an initial low dose of cocaine (10 mg/kg, i.p.). This classification has been found to predict cocaine's inhibition of the dopamine transporter (DAT; HCRs > LCRs; Sabeti et al., 2002), DAT function within dorsal striatum (dSTR; HCRs > LCRs; Briegleb et al., 2004; Mandt and Zahniser, 2010), and cocaine-induced locomotor sensitization and conditioned place preference (LCRs > HCRs; Allen et al., 2007; Mandt et al., 2008; Sabeti et al., 2003). Thus, it appears that LCRs may represent a phenotype for increased susceptibility to cocaine's "addictive" properties. Furthermore, one previous study found LCRs to exhibit greater "motivation" than HCRs to self-administer a range of cocaine doses (Mandt et al., 2008). Surprisingly, however, LCRs and HCRs did not differ in the acquisition of cocaine self-administration in that study. One possible explanation is that the conditions used (e.g. cocaine dose) were not appropriate to reveal LCR/HCR differences in acquisition. Thus, the present study further investigated the role of LCR/HCR classification on the acquisition of self-administration reinforced by higher cocaine

doses (0.375, 0.5 and 1 mg/kg/inf) according to a fixed-ratio 1 schedule of reinforcement and tested the hypothesis that LCRs, more readily than HCRs, would acquire higher dose cocaine self-administration. No LCR/HCR differences were revealed in the acquisition of 0.375 or 0.5 mg/kg cocaine self-administration. There was a statistical trend for LCRs to more readily acquire self-administration of 1 mg/kg cocaine relative to HCRs ($p=0.06$). Further, LCRs, more so than HCRs, exhibited dose-dependent effects in the latency to and percentage of acquisition of self-administration for the cocaine doses tested here. These data suggest that a higher dose of cocaine (1 mg/kg/inf) functions as a more effective reinforcer for LCRs than for HCRs and adds to evidence that LCRs represent a phenotype for increased susceptibility to cocaine's "addictive" properties.

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EOSINOPHIL REDUCTION ATTENUATES REMODELING IN SAMP1 MICE

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Background: The senescence-accelerated (SAMP1) mouse strain develops spontaneous ileitis and recapitulates the pathology of human Crohn's disease. Further examination of ileal tissue from this strain reveals significant eosinophilic infiltration and remodeling.

Purpose: We hypothesize that eosinophilic infiltration contributes to intestinal tissue remodeling. The aim of this study was to characterize the impact of \square CCR3 and \square IL-5 antibody treatment on eosinophilic infiltration and tissue remodeling in the SAMP1 model of Crohn's-like ileitis.

Description: Eosinophil inhibition studies were performed with single or combined \square CCR3 and/or \square IL-5 antibody injections during the chronic stage of disease, from 20 to 30 weeks, in SAMP1 mice. Flow cytometry for lamina propria leukocytes including eosinophils was performed. Histological features of ileitis in SAMP1 mice were assessed using H&E and Periodic Acid Schiff staining. Real Time PCR analysis was performed on ileal tissue for the TGF- \square family, epithelial and mesenchymal markers of remodeling, collagen and extracellular matrix components, remodeling associated proteases, eotaxins, their receptors and mucin genes. All assessments were compared to age-matched control AKR mice.

Results: Blockade of lamina propria eosinophil infiltration was observed following 10-weeks of antibody infusion (\square CCR3 & \square IL-5; $p < 0.001$) and resulted in attenuation of all indices of histological ileal inflammation (villus distortion, chronic and active inflammatory infiltrates) (\square IL-5; $p < 0.01$, \square

CCR3 & \square IL-5; $p < 0.01$). Cellularity of both the spleen and reactive draining mesenteric lymph nodes were significantly reduced by combined antibody therapy ($p = 0.03$). Furthermore, flow cytometric analysis revealed a reduction in lamina propria central memory T-lymphocytes ($CD4^+ CD44^{high} CD62-L^{high}$). Reduction in tissue remodeling was also observed, as demonstrated by reduced muscle hypertrophy (\square CCR3; $P < 0.05$, \square IL-5; $p < 0.01$, \square CCR3 & \square IL-5; $p < 0.001$) and reduced goblet cell hyperplasia (\square CCR3; $P < 0.01$, \square IL-5; $p < 0.101$, \square CCR3 & \square IL-5; $p < 0.001$). The fibroblast chemo-attractant and eosinophil binding protein fibronectin, was the most increased gene during the progression of remodeling in these mice (20 fold; $p < 0.0001$ at 20 weeks-of-age, 30 fold; $p = 0.0007$ at 40 weeks-of-age vs. age matched control AKR mice) and was significantly decreased following antibody treatment (\square CCR3; $p < 0.01$).

Conclusions: SAMP1 mice demonstrate Crohn's-like ileitis with histological and molecular features of eosinophilic infiltration and tissue remodeling. Antibodies against CCR3 and IL-5 are effective in reducing eosinophil infiltration, overall inflammation and tissue remodeling. We anticipate that this model will provide a valuable tool for further elucidating the eosinophil's role in the pathogenesis of inflammatory bowel diseases.

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**ALUMINUM VACCINE ADJUVANTS
ACTIVATE THE NLRP3 INFLAMMASOME
BUT THIS PATHWAY IS NOT REQUIRED
FOR ENHANCED T CELL PRIMING OR
ANTIBODY RESPONSES**

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Background: Aluminum salts (alum) are particulate adjuvants used widely in human subunit vaccines to enhance antibody responses against soluble antigens. Such adjuvants have been used in vaccines for almost a century, and historically their effects have been attributed to the formation of long lived antigen depots. However, more recent studies have shown that alum initiates recruitment of monocytes to the injection site and that these cells develop into dendritic cells that present antigen in the draining LN. Purpose of study: Alum induces activation of the NLR family, pyrin domain containing 3 (NLRP3) inflammasome and release of IL-1beta in vivo, however the requirement for this pathway in alum's adjuvant effects is controversial. The purpose of our study was to determine which innate cells release IL-1 in response to alum adjuvants in vivo and to determine the role that these cells and the NLRP3 inflammasome play in promoting T cell responses. Methods used: We tracked the innate response to alum in vivo using flow cytometry and detected cytokine release by ELISA. T cell responses to injected antigen were tracked ex vivo using MHC I and MHC II tetramers and flow cytometry. Results: Our observations indicate that in vivo, alum induces cytokine production from macrophages and mast cells via NLRP3 dependent and independent pathways. These sensor cells promote the inflammatory response to alum, but are not required for the effects of alum on enhancing T cell priming or for promotion of enhanced antibody responses. In mice that lack NLRP3 or caspase-1, we observed reduced levels of IL-1beta following immunization with alum, but observed no impact on T cell priming or antibody production. Conclusions: These results suggest that although alum activates the NLRP3 inflammasome in vivo, it is not required for alum's effects as an adjuvant. Current work

is focused on identifying alternative or redundant pathways by which alum adjuvants impact antigen presentation and promote T cell priming.

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TIM-3 EXPRESSION ON PD-1^{POS} HEPATITIS C VIRUS-SPECIFIC CD8+ T CELLS PREDICTS PERSISTENCE IN ACUTE INFECTION AND ITS BLOCKADE RESTORES CYTOTOXICITY

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Background: Hepatitis C virus (HCV) establishes persistent infection in ~75 to 80% of patients. HCV has successfully developed mechanisms to evade immune clearance including interfering with the virus-specific T cell response. The function of CD8+ T cells can be inhibited by ligation of inhibitory receptors on the cell surface. We hypothesized that the co-expression of the negative regulatory receptors, T cell immunoglobulin and mucin domain-containing molecule 3 (Tim-3) and programmed death 1 (PD-1) on T cells in HCV infection would identify patients at risk of developing viral persistence during and after acute infection.

Results: We studied 81 patients with HCV, including acute HCV infection at two time points (11 acute to resolved, 14 acute to chronic), 33 treatment naïve patients who had chronic HCV for more than 10 yrs and 23 subjects who had spontaneously resolved HCV in the remote past. The expression of Tim-3 was statistically higher on both CD4+ and CD8+ T cell subsets in acutely infected patients who failed to clear the virus ($p = .0047$, $p = .0002$, respectively). In addition we examined the dual expression of both PD-1 and Tim-3 on HCV-specific cytotoxic T lymphocytes (CTLs). At all time points studied, patients who resolved HCV infection demonstrated lower frequencies of dual Tim-3⁺/PD-1⁺ HCV-specific CTLs than double negative HCV-specific CTLs. The population of PD-1⁺/Tim-3⁺ T cells displayed a central memory phenotype and higher expression levels of these inhibitory molecules correlated with diminished Th1/Tc1 cytokine secretion. Furthermore, while blockade of either PD-1 or Tim-3 enhanced proliferation of HCV-specific CTLs, T cell cytotoxicity against a hepatocyte cell line that expressed the cognate HCV epitope was increased exclusively by Tim-3 blockade.

Conclusions: Taken together, these data indicate that defective T cell responses, the

primary reason for lack of immune control of persistent pathogens such as HCV, track with the expression of these inhibitory molecules, and their manipulation represent rationale targets for novel immunotherapeutic approaches.

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ROLE OF ISLET1 IN THE ELECTRICAL DEVELOPMENT OF SPINAL NEURONS

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In murine, avian and amphibian systems, motor neuron electrical development follows a stereotypic program involving increases in outward and inward current amplitudes and consequent shortening of action potential durations. In previous studies, motor neurons have been studied as a single population. However, genetic and morphological properties clearly reveal different motor neurons subtypes. Consequently, it is not known whether motor neuron subtypes develop identical or different electrical properties. To address this question, we have characterized electrical development of zebrafish spinal cord primary motor neuron subtypes (PMNs) that are accessible and distinguishable even prior to axonal outgrowth.

We focused on two PMN subtypes; CaP (caudal) and MiP (middle). These PMNs are identifiable by their unique expression of transcription factors, soma position, axonal trajectories and muscle targets. Our findings demonstrate that CaP and MiP differ with respect to electrical properties prior to the development of axonal projections and suggest that different intrinsic programs drive early electrical differentiation of each PMN subtype.

What molecules direct development of early electrical properties in motor neurons? Each PMN subtype expresses a unique set of LIM-homeodomain (HD) transcription factors. Among these factors, Islet1 is expressed by all PMNs as they exit the cell cycle. Further, Islet1 plays an important role in motor neuron morphological differentiation. Taking advantage of the ease of morpholino antisense methods in zebrafish, we knocked-down Islet1 to determine its role in motor neuron electrical differentiation. Our findings suggest that Islet1 dependent pathways participate in the electrical development of motor neurons.

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THE MOLECULAR BASIS OF HISTONE BINDING BY THE CHD4 PHD FINGERS

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CHD4 is an ATPase and a major subunit of the NuRD (nucleosome remodeling and deacetylase) complex, which is involved in transcriptional regulation and development. It is also an autoantigen of the disease Dermatomyositis (DM), an idiopathic inflammatory disorder of the muscles, which leads to an increased risk of cancer and heart disease. CHD4 contains a tandem of plant homeodomain (PHD) fingers, two chromodomains and an ATPase module. The PHD construct is found in many chromatin remodeling complexes and transcription factors, and is often found to be important for the targeting of these complexes to chromatin. Here we determine the specificity of the individual PHD domains of CHD4 for the unmodified tail of histone H3 and show that this interaction is modulated by covalent modification of Lys4 and Lys9. We use NMR, mutational analysis and modeling to characterize the molecular basis of the PHD2 interaction, which binds over 40-fold stronger than PHD1. Analysis of the tandem PHD construct further reveals that the PHD fingers act synergistically when they are linked, increasing the strength of the H3 interaction by over 10-fold, and suggesting a multivalent targeting of CHD4 to chromatin.

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GENOMIC RECONSTRUCTION OF THE EVOLUTIONARY HISTORY OF DUF1220 DOMAINS.

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With the mounting number of eukaryotic genomes available for comparative studies, there is an emerging appreciation for the role that copy number variations (CNVs) can play in evolution and disease. While most studies have focused on segmental duplications/deletions of substantial size (>20 kb), small-sized CNVs have been, by comparison, only minimally investigated. One of the most evolutionarily striking copy number variations (CNVs) that has been identified to date is the dramatic human lineage-specific amplification of a 65 amino acid protein domain of unknown function, DUF1220. Sequences encoding DUF1220 domains are increasingly amplified generally as a function of a species evolutionary proximity to humans, where the greatest number of copies is found (218). DUF1220 domains are virtually all primate specific and are typically found within the NBPF (neuroblastoma break point factor) gene family. Recently 1q21.1 CNVs that either encompass or flank DUF1220 domains have been implicated in a number of human diseases, including microcephaly, macrocephaly, autism, schizophrenia, mental retardation and heart disease, suggesting a link between DUF1220 amplification and the etiology of these disorders.

The recent availability of numerous mammalian genome sequences presents an opportunity to investigate the evolutionary history of DUF1220 domain amplifications in unprecedented detail. To accomplish this available mammalian genome sequences were interrogated and all DUF1220 domains and the genes in which they are found were identified. The results reported here support previous findings that DUF1220 copy number is highest in the human lineage. Interestingly previously unidentified NBPF genes were found in several non-primate mammalian genomes (only recently made available and heretofore not known to contain NBPF genes), and a newly identified non-primate mammalian conserved promoter region, upstream of several NBPF genes, was also identified. Calculation of

DUF1220 numbers and locations within the various genomes supports the view of not only extreme amplification in the primate and especially human lineage, but also of increasing NBPF complexity both in gene structure and expression. These new findings represent the most complete picture of DUF1220 evolution so far reported and lend further support to the view that there have been strong selection pressures that favored the rapid copy number expansion of these domains over recent evolutionary time.

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CONTRIBUTION OF THE PRRH REGULATORY RNA TO HEME REGULATION IN *PSEUDOMONAS AERUGINOSA*

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The opportunistic pathogen *Pseudomonas aeruginosa* requires iron and employs several strategies for its acquisition, including the uptake of heme, an abundant source of iron in the human host. *P. aeruginosa* expresses at least two outer membrane (OM) heme receptors, PhuR and HasR, which transport heme into the periplasm. A periplasmic (PP) heme binding protein, PhuT, can then shuttle heme to an inner membrane (IM) permease, PhuUV, which transports heme into the cytoplasm. While necessary for survival, iron and heme can be toxic; thus, iron and heme acquisition are tightly controlled by intracellular iron concentrations via the Fur protein. In iron-replete conditions, Fur binds to the promoters of genes for iron and heme acquisition, blocking their expression. Fur also represses expression of two nearly identical regulatory RNAs encoded in tandem, PrrF1 and PrrF2, which are thought to contribute to iron homeostasis by controlling key metabolic processes. We recently described a third RNA encoded by the *prfF* region, transcription of which initiates at the *prfF1* promoter, extends through the *prfF1-prfF2* intergenic region, and terminates at the *prfF2* terminator. This 325-nucleotide RNA, designated PrrH, is repressed by heme via an unknown mechanism. Currently, we are working to understand the mechanism of this regulation, as well as the biological role of this novel RNA. Here, we show that the PhuR and HasR OM receptors contribute to heme repression of PrrH, while the PP heme binding protein, PhuT, and IM heme permease, PhuUV, are dispensable for this regulation. Combined, these data support a model in which heme initiates a signaling cascade that leads to PrrH repression either by binding to or translocation via the PhuR and HasR OM receptors. To determine the biological role of PrrH, we identified putative PrrH-specific target mRNAs by searching for complementarity to the unique region of PrrH. Several of these mRNAs are responsive to heme and derepressed upon deletion of the

prfF region, indicating PrrH plays a role in this regulation. Overall, these studies begin to address the role that PrrH plays in mediating *P. aeruginosa*'s response to heme.

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**THE EFFECT OF ORF1 PROTEIN
MUTATIONS ON L1 RETROTRANSPOSITION**

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L1 is a ubiquitously distributed non-long terminal repeat retrotransposon in mammals that constitute ~17% of human and ~19% of mouse genome. The majority of L1s are unable to retrotranspose because they are 5' truncated, mutated or rearranged, but a few L1s are actively retrotransposed and can cause diseases. L1 transposes autonomously using its self-encoded protein machinery from two non-overlapping open reading frames (ORFs). The protein encoded by ORF1 acts as an RNA binding protein and is also a nucleic acid chaperone although its precise role in retrotransposition is unknown. The purpose of my study is to understand the role of ORF1 protein in L1 retrotransposition by mutational analysis.

ORF1 protein contains three structural domains: a coiled-coil (C-C) domain, an RNA recognition motif (RRM) domain, and a C-terminal (CTD) domain. Atomic force microscopy (AFM) and analytical ultracentrifugation showed that the purified mouse ORF1p adopts a dumbbell-shaped, homotrimeric structure. The role of ORF1 protein during L1 retrotransposition has been investigated by introducing amino acid substitutions in to the C-C, RRM and CTD domains; the mutations are K176A, R190A, R238A/K, R251A/K, R284A/K and Y318A/F. L1 retrotransposition was analyzed by an autonomous retrotransposition assay (ART), and steady-state levels of ORF1 protein and L1 RNA were quantified across the time course of L1 retrotransposition.

The ART assay results showed that the K176A and Y318F mutations had no effect on retrotransposition as both were transposed with efficiency similar to WT. R190A and R284K showed about 50% reduction in the assay. The mutants R238A/K, R251A/K, R284A, and Y318A had no measurable retrotransposition activity in the ART assay. In spite of these large differences in retrotransposition activity, all of these mutants expressed near wild-type levels of L1 RNA and ORF1p throughout the time course indicating that lower or loss of

retrotransposition is not due to the loss of ORF1 protein or L1 RNA. To further understand the basis for the loss of retrotransposition activity in the various ORF1 mutants, experiments to trap and assay retrotransposition intermediates are in progress.

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LOSS OF SLAP EXPRESSION IN SKG MICE LEADS TO THE PREVENTION OF CHRONIC ARTHRITIS BY ENHANCING TREG NUMBERS

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Background: Intrinsic defects in lymphocyte signaling have been implicated in the pathogenesis of rheumatoid arthritis (RA) by recent genome wide genetic association studies, as well as a mouse model of inflammatory arthritis (SKG). SKG mice have a spontaneous mutation in the zeta-chain-associated protein kinase of 70kDa (ZAP-70) that leads to impaired thymocyte development, reduced positive selection and impaired negative selection of thymocytes. These defects result in autoreactive peripheral T cells that drive a condition resembling RA. SLAP has been shown to influence TCR levels and signaling in developing thymocytes and activated peripheral T cells. We hypothesized that the loss of SLAP function could enhance TCR complex-mediated signaling and restore normal T cell development and function.

Results: To test this hypothesis we generated Double SKG SLAP KnockOut (DSSKO) mice. Upon zymosan challenge, which induces arthritis in SKG mice, DSSKO mice do not develop chronic arthritis. Thymocytes from DSSKO mice have increased TCR complex mediated signaling as evidenced by increased surface expression of CD5 and ERK phosphorylation. Furthermore, DSSKO thymocytes do not have enhanced mammary tumor virus-superantigen mediated deletion suggesting that the loss of SLAP function is not leading to significant alteration in thymocyte negative selection. Instead, DSSKO mice have a 2-fold increase in thymic and peripheral FOXP3+ Tregs compared to SKG controls. Peripheral CD4+ T cells from DSSKO mice treated with zymosan had decreased percentages of TH17 cells and an expansion in the numbers of Tregs compared to SKG mice. CD25 depletion of zymosan-treated DSSKO mice leads to the development of arthritis, which supports the hypotheses that the increase in Tregs seen in DSSKO mice has a dominant role in the arthritis amelioration.

Conclusions: These data support the model that loss of SLAP function in SKG mice enhances the development and expansion of Tregs, which leads to arthritis amelioration. These studies lead to novel insights into pathogenesis of arthritis in SKG mice. Our ongoing work is defining the mechanism of Treg development, expansion and function in DSSKO mice, which may ultimately lead to new ways of modulating Treg numbers to treat autoimmune disease.

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CHARACTERIZATION OF MICRO-RNA EXPRESSION IN SECRETORY ACTIVATION

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MicroRNA (miRNA) expression in the postnatal mouse mammary gland has been recently investigated by microarray profiling of whole mammary gland (1). However, because miRNA expression is known to be highly cell-type specific, we performed both mRNA and miRNA expression profiling on isolated mammary epithelial cells (MECs) from pregnancy day 14 (P14) and lactation day 2 (L2). Four CD1 mice were used for each time point. We hypothesized that miRNA might control some aspects of secretory activation such as glycolysis and lipid synthesis pathways. Affymetrix GeneChip® Mouse Gene 1.0 ST Array and GeneChip® miRNA Array were used for hybridization according to the manufacturer's methods.

Statistical analysis of the mRNA changes between P14 and L2 identified 5,499 unique genes as being differentially expressed (5% FDR); of these 2,902 genes and 2,604 genes were higher in P14 or L2 stages, respectively. Genes upregulated at L2 included most of the critical mediators of secretory activation, namely milk protein and lipid synthesis genes.

We identified 69 miRNA transcripts differentially expressed (DE) between P14 and L2 stages (20% FDR); of these, 32 miRNAs showed greater than two-fold change. Predicted miRNA targets for the 32 miRNAs were obtained from three target prediction algorithms: TargetScan, miRanda and miRDB algorithm. The intersection of this gene list with that of the differentially expressed (DE) mRNA in the same cells showed that approximately 49% of these mRNA genes were predicted targets of DE miRNA. Amongst the putative targets of miRNA decreasing between P14 and L2, are 765 genes upregulated in lactation. Additionally, 5 out of 6 mature miRNAs lying within the miR-17~92 cluster located on Chr14 are significantly upregulated at P14. This cluster is highly conserved in mammals and implicated in both development and tumorigenesis (2). Predicted targets of this

cluster alone can potentially regulate 27% of the genes upregulated in lactation, including 25 genes in the glycolysis and lipid synthesis pathways.

Similar expression patterns and fold changes were observed for 4 genes and 4 miRNAs selected for validation of array data by quantitative RT-PCR. Functional validation of selected miRNAs was performed using antagomirs in an *in vitro* mammary epithelial cell line - CIT3 cells and results reveal that miR-15b inhibits lipid synthesis by regulating fatty acid synthase (Fasn) and glycerol kinase (Gyk) mRNA and protein in mammary epithelial cells.

References:

1. Avril-Sassen, S., and Caldas, C. (2009) *BMC Genomics*. **10**, 548.
2. Mendell, J. T. (2008) *Cell*. **133**, 217-222.

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ANALYSIS OF FUNCTIONAL DOMAINS IN MI-2/NURD NUCLEOSOME REMODELING/HISTONE MODIFYING COMPLEXES

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Mi-2/nucleosome remodeling and deacetylase (Mi-2/NuRD) chromatin remodeling complexes (CRCs) perform multiple regulatory functions in the nucleus, including histone deacetylation and demethylation, nucleosome mobilization and recruitment of transcriptional co-repressors/co-activators. Mi-2 β (or chromodomain helicase DNA-binding protein 4, *CHD4*), a core catalytic component of Mi-2/NuRD complexes, is the primary autoantigen associated with adult dermatomyositis. This autoimmune disease substantially increases the risk of developing cancer. Mi-2 β is a 218 kDa SNF2-like ATPase comprising several functional domains: two plant homeodomain (PHD) zinc fingers, two chromodomains, an ATP-binding helicase domain, a DEAH box and a C-terminal domain that differs extensively between SNF2 family members. However, the function(s) of Mi-2 β 's various domains remain largely unknown. We have developed an in vitro assay that allows knock-down of endogenous Mi-2 β in plasmacytoma cells. Depletion of Mi-2 β results in dramatic transcription factor-dependent activation of endogenous *CD79a/mb-1* promoters. This process is dependent on SWI/SNF and efficient demethylation of *CD79a/mb-1* promoter DNA. Here, we show that add-back of wild-type Mi-2 β in cells concurrent with endogenous Mi-2 β knock-down restores the repression of *CD79a/mb-1* transcription. 'Add-back' of an ATPase-dead mutant (K757R) Mi-2 β failed to mediate repression. To identify functions of other domains, we generated mutations in each of Mi-2 β 's two PHD fingers, which mediate interactions with histone H3. Studies will focus on the importance of these sequences for Mi-2 β DNA binding, promoter accessibility, histone binding and facilitation of DNA demethylation. Our studies will achieve a comprehensive understanding of how Mi-2/NuRD mediates

diverse functions associated with gene activation, repression and oncogenesis.

A NOVEL MOUSE MODEL FOR STUDYING TRAFFICKING OF ENDOGENOUS DOPAMINE TRANSPORTER

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Dopamine (DA) transporter (DAT) is a transmembrane protein that functions at plasma membrane of DA neurons to clear extracellular DA, thus causing termination of DA neurotransmission. DAT is linked to several neurological disorders with abnormal DA neurotransmission such as Parkinson's disease, schizophrenia, attention deficit hyperactivity disorder, and drug addiction. DAT participates in DA clearance only when it is present on the plasma membrane and surface DAT levels are regulated by endocytic trafficking. But the mechanism of constitutive and stimuli-induced (for example psychostimulant drugs) DAT endocytic trafficking is poorly understood. Most of the current knowledge on regulation of DAT trafficking is based on studies done in non-neuronal cell systems, and therefore, the mechanism of DAT trafficking in DA neurons remains unclear. To better understand DAT endocytosis in DA neurons, our laboratory has developed knock-in mice expressing functional DAT with a hemagglutinin (HA) epitope in the DAT second extracellular loop (HA-DAT). In mesencephalic neuronal cultures derived from these mice, HA-DAT was found to localize to tyrosine hydroxylase (a marker for DA neurons) positive neurons. Our laboratory has previously demonstrated that introduction of the HA11 tag in human DAT expressed in PAE cells does not perturb protein function. Likewise, mice homozygous for the HA-DAT knock-in allele showed normal DAT expression levels and normal DA uptake in striatal synaptosomal preparations. This demonstrates that these mice are functionally normal and can serve as a functional tool for further studies of DAT *in vivo*.

The HA-tag equips us with an ability to monitor DAT movements in live DA neurons. In primary DA neuronal cultures derived from these mice, Fluorescence Recovery After Photobleaching (FRAP) experiments demonstrated that DAT in the axonal varicosities (active synapses) have a higher diffusion co-efficient than DAT associated with axonal extensions (extrasynaptic). Single

Particle Tracking (SPT) were also performed which demonstrated that a certain population of DAT is nocodazole-sensitive. Endocytosis assays using HA-antibody feeding technique demonstrated that HA-DAT undergoes constitutive and stimuli-induced endocytosis mediated by amphetamine and phorbol esters (PMA). Our future experiments are aimed at using these techniques to further elucidate the mechanisms of constitutive as well as stimuli-induced DAT endocytic trafficking in DA neurons.

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THE ROLE OF TNF- α AND MACROPHAGES IN THE RESOLUTION OF PULMONARY FIBROSIS.

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Idiopathic pulmonary fibrosis/usual interstitial pneumonia (IPF/UIP) is a progressive, fibrotic interstitial lung disease for which there is no known cause or effective therapy. The pathogenesis of IPF/UIP is associated with marked increases in the abundance of myofibroblasts and collagen. We hypothesize that myofibroblast apoptosis requires a conditional sequence of signals initiated by macrophage-derived TNF- α and induced by ligation of Fas. In these studies we address two questions. 1) Is endogenous macrophage production of TNF- α sufficient to induce Fas-ligand-mediated apoptosis of primary pulmonary fibroblasts *in vitro* and 2) is induction of *in vivo* TNF- α production a viable treatment to promote the resolution of pulmonary fibrosis? TNF- α production by bone marrow derived macrophages (BMM Φ) after LPS stimulation was found to be sufficient to induce Fas-ligand mediated apoptosis of primary fibroblasts as determined by positive caspase-8 staining. Loss of TNF- α signaling through antibody neutralization of TNF- α or by genetically deficient mice with the loss of TNF- α production (TNF- $\alpha^{-/-}$) by BMM Φ or the TNF- α receptor (TNF-R1 $^{-/-}$) in pulmonary fibroblasts confirms that TNF- α signaling is also necessary to induce apoptosis. Using the murine model of bleomycin-induced pulmonary fibrosis, TNF- α was administered by intratracheal injection either early in the disease progression or after fibrosis was established. A reduction in fibrosis, as determined by changes in pressure-volume curves, increased static compliance, decreased inflammatory cell infiltration, and decreased hydroxy-proline, was observed after TNF- α administration. In addition, depletion of pulmonary macrophages using hyperchlorinated water during the induction of fibrosis increases disease severity based on the same criteria. Understanding the role of macrophage derived TNF- α in the resolution of pulmonary fibrosis will provide new insights for how myofibroblast apoptosis is regulated by the inflammatory process, how this event becomes impaired in IPF/UIP, and how

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this knowledge can be applied to reverse the accumulation of these cells in the lungs of patients with IPF/UIP.

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ECTODERMAL WNT/ β -CATENIN SIGNALING IS REQUIRED TO SHAPE THE MOUSE FACE

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Recent studies have identified the FEZ (frontonasal ectodermal zone) as an important signaling center that regulates growth and patterning of the vertebrate face. The FEZ expresses *Fgf8* and *Shh* and manipulating the domain of these morphogens has been shown to alter the shape of upper and midface morphology in a chick model. Thus, it has been hypothesized that changes in FEZ organization may drive evolutionary changes in facial shape. In the present study, we show that genetic manipulation of ectodermal Wnt/ β -catenin signaling in the mouse face using *Cre*, a novel ectodermal Cre recombinase, alters growth of the mouse face. Loss of β -catenin in the ectoderm results in a profound reduction of lateral facial growth inducing a beak-like structure more characteristic of an avian face than a mouse. This phenotype is concomitant with a loss of *Fgf8* expression. Conversely, constitutive activation of β -catenin signaling in the ectoderm causes a dramatic expansion of *Fgf8*, as well as ectopic *Fgf4* expression, leading to an increase in lateral facial growth and a wider face. Together, these results demonstrate that ectodermal β -catenin controls facial growth most likely through alteration of FEZ-associated signaling molecules. Our data support a critical role for ectodermal Wnt/ β -catenin signaling during craniofacial development, and further suggest that changes in this signaling pathway may have been important during the evolution of diverse facial shapes present in vertebrate species.

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THE BH3-MIMETIC ABT-737 INDUCES STRONG SYNERGISTIC KILLING OF MELANOMA CELLS WHEN COMBINED WITH THE ALKYLATING AGENT TEMOZOLOMIDE.

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Metastatic melanoma has poor prognosis and is refractory to most conventional chemotherapies. The alkylating agent temozolomide (TMZ) is commonly used in treating melanoma but has a disappointing response rate. Agents that can act cooperatively with TMZ and improve its efficacy are thus highly sought after. One possible agent is the BH3 mimetic ABT-737, which can induce apoptosis by targeting pro-survival Bcl-2 family members. Yet, research has shown that many cell lines are resistant to ABT-737 via the upregulation of the pro-survival protein Mcl-1 and/or the down-regulation of the pro-apoptotic protein Noxa, which can inhibit Mcl-1. We found that a combination of TMZ and ABT-737 induced strong synergistic apoptosis in multiple human melanoma cell lines, while inducing little or no apoptosis in normal melanocyte lines. However, sensitivity to the drug combinations was variable, and two melanoma cell lines were completely resistant. Further study revealed that resistance was strongly correlated with the expression of MGMT and PARP, two proteins involved in TMZ resistance by known mechanisms. Inhibition of these proteins via O⁶-benzylguanine and ABT-888, respectively, reduced or eliminated resistance to the drug combination. Furthermore, these two inhibitors worked together synergistically to enhance sensitivity. Immunoblot analysis and experiments with knockdown cell lines suggested that TMZ and ABT-737 synergy is mediated through upregulated Noxa. Our results show that TMZ and ABT-737 are a promising drug combination for clinical studies, particularly when combined with pretreatment by MGMT and PARP inhibitors.

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**STRUCTURAL INSIGHT INTO P53
RECOGNITION BY THE 53BP1 TANDEM
TUDOR DOMAIN**

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The tumor suppressor p53 and DNA repair factor 53BP1 regulate gene transcription and responses to genotoxic stresses. Upon DNA damage, p53 undergoes dimethylation at Lys382 (p53K382me₂), and this posttranslational modification is recognized by 53BP1. The molecular mechanism of the nonhistone methyllysine mark recognition remains unknown. Here we report a 1.6 Å resolution crystal structure of the tandem Tudor domain of human 53BP1 in complex with a p53K382me₂ peptide. In the complex, dimethylated Lys382 is restrained by a set of hydrophobic and cation- π interactions in a cage formed by four aromatic residues and an aspartate of 53BP1. The signature HKKme₂ motif of p53, which defines the specificity, is identified through a combination of NMR resonance perturbations, mutagenesis, measurements of binding affinities and docking simulations and analysis of the crystal structures of 53BP1 bound to p53 peptides containing other dimethyllysine marks, p53K370me₂ and p53K372me₂. Binding of the 53BP1 Tudor domain to p53K382me₂ may facilitate p53 accumulation at DNA damage sites and promote DNA repair as suggested by chromatin immunoprecipitation and DNA repair assays. Together, our data detail the molecular mechanism of the p53-53BP1 association and provide the basis for deciphering the role of this interaction in regulation of p53 and 53BP1 functions.

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MICE LACKING AKAP150-CALCINEURIN ANCHORING HAVE IMPAIRED HIPPOCAMPAL LTD.

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Protein kinases and phosphatases are emerging as key molecular components in synaptic plasticity underlying learning and memory in hippocampal neurons. A-kinase-anchoring protein (AKAP) 79/150 (79 human/150 rodent) anchors both PKA and calcineurin (CaN) and is linked to NMDA and AMPARs through MAGUK scaffolding proteins. The AKAP complex is thought to be involved in LTD by regulating AMPAR localization at excitatory synapses by regulating the opposing actions of PKA and CaN. LTD, induced through a low frequency stimulation, leads to a decrease in synaptic AMPAR activity and number, by rapid dephosphorylation and/or endocytosis of the receptor. Both mechanisms are thought to require the enzymatic activities of PKA and CaN. Previous work in our lab identified a conserved PxlIT-like anchoring motif in the c-terminal region of AKAP 79/150 (AKAP150 631PIAIIIT637), and that AKAP 79/150-CaN anchoring opposes PKA regulation of neuronal L-type calcium channels. Here, we used a novel AKAP150 Δ PIX knock-in mouse to investigate the role of AKAP79/150 CaN anchoring in regulating AMPARs during induction of NMDAR dependent LTD at synapses between hippocampal CA3-CA1 neurons. Hippocampal slices (400 μ m) were prepared from 2-3 week old wild type (WT), or Δ PIX mice. LTD was induced in the hippocampal CA1 region by low-frequency stimulation (LFS; 1 Hz stimulation of Schaffer collaterals/commissural fibers for 900 s, 15 min), and fEPSP amplitudes and slopes were measured in the stratum radiatum for one hour. LTD was significantly decreased in AKAP150 Δ PIX mice compared with WT control mice. Δ PIX mice exhibited enhanced LTP (100 Hz) as compared to WT control. Data from cultured hippocampal neurons using chemically induced LTD (NMDA-induced) revealed impaired removal of AMPARs from synapses in AKAP150 Δ PIX mice. Future experiments will

utilize whole-cell electrophysiology techniques to determine if basal NMDA and AMPAR synaptic activity levels are altered in these AKAP150 Δ PIX mice.

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LIPID SYNTHESIS AS A SURVIVAL MECHANISM FOR CANCER CELLS

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Fat synthesis in cancer cells can help cancer cells grow and survive breast cancer treatments. The female hormone progesterone is essential for pregnancy and also plays a role in weight gain and breast cancer risk. One effect of progesterone on breast cancer cells is that it induces a subset (~20%) of cells to make fat (lipid) droplets. By examining the breast cancer cells that contain fat droplets, we hope to identify molecular markers that can be used to design novel therapeutic cancer killing drugs. Our goal is to study the behavior of breast cancer cells that contain lipid droplets vs. those that do not contain lipid droplets.

Hypothesis: We speculate that fat-loaded breast cancer cells have altered growth and enhanced resistance to cancer-killing drugs compared to non-fat loaded cancer cells.

Methods: Live cell and fluorescence microscopy were used to examine T47Dco breast cancer cells stimulated with synthetic progesterone (MPA) or vehicle (EtOH). After 4 days of MPA or EtOH incubation, hormone treatment was continued and cells were exposed to the chemotherapy drug docetaxel (Dx) or its vehicle (DMSO) for 2 additional days. Bromodeoxyuridine and adipophilin staining were used to assess proliferation and lipid accumulation respectively. DAPI was used to stain the nuclei. Glucose uptake was measured using H^3 2DeoxyGlucose. Intracellular lipids were analyzed by thin layer chromatography (TLC).

Results: MPA +DMSO caused 12% of breast cancer cells to become lipid loaded while none of the EtOH+DMSO treated cells contained lipid droplets. Of the total population of cells treated with MPA+DMSO ~20% were proliferative, however, among cells that contained lipid droplets only ~5% of cells were proliferative. Cells treated with MPA+Dx showed a ~5 fold lower proliferation than cells treated with MPA+DMSO. MPA+Dx also caused an increase in cells containing lipid droplets (~19% of cells versus 12% of cells treated with MPA+DMSO), however, very few cells containing lipid droplets were proliferative. MPA also caused a 50%

increase in glucose uptake that was independent of insulin. Fatty acids and free cholesterol were increased in response to MPA treatment by TLC analysis.

Conclusions: Increased lipid synthesis in breast cancer cells exposed to MPA is associated with increased glucose uptake, increased proliferation and protection from docetaxel drug treatment. We speculate that the carbons from the increased glucose uptake may be used to make more lipids in MPA-treated cells, providing survival mechanisms for cancer cells exposed to cancer-killing drugs. Identifying genes specifically expressed in the fat-loaded cells will allow us to elucidate new drug targets to increase the efficacy of breast cancer treatments.

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PRENATAL STRESS IMPAIRS MEMORY FOR NOVEL OBJECT SPATIAL LOCATIONS IN ADULTHOOD.

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Exposure to stress during gestation has been linked with psychopathologies in humans ranging from depression and anxiety to schizophrenia. Although the hallmark of schizophrenia is psychosis, memory deficits are also common and are often the best predictors of psychosocial functioning and negative outcomes in daily life. The current study investigated the effects of prenatal stress on memory for novel spatial locations in adulthood. 20 pregnant female Sprague-Dawley rats experienced unpredictable variable stress 2-3 times daily during the last week of gestation (14-21). At weaning (21 days) the offspring of the prenatally stressed (PNS) and nonstressed (NS) dams were assigned to same-sex PNS or NS groups (male vs. female, PNS vs. NS; n=10; overall N=40). In adulthood (77-87 days of age), all animals were acclimated to the test arena for 5 min/day for a total of 4 days. Animals received only one memory test per day, for a total of 3 novel spatial location tests. Testing consisted of an acquisition phase in which animals explored two identical novel objects for 5 minutes, and a test phase (1 hour later) in which one of the previously explored objects was moved to a new spatial location. Rats that remember the previous spatial location of objects will spend more time investigating the object in the new spatial location. A 2-factor (condition x sex) between subjects repeated measures (test day) ANOVA was used to analyze the number and duration of visits made to the familiar and novel object locations during the 30 seconds following placement in the test arena. NS animals spent a significantly greater proportion of time investigating the novel location (relative to the familiar location) than did PNS animals [Figure 1A; $F(1,42)=4.94$, $p=0.037$]. NS animals also showed a higher proportion of visits to the novel object location than did PNS animals [Figure 1B; $F(1,42)=11.41$, $p=0.0028$]. No significant effects of sex or test day were detected in this

analysis. These results are consistent with previous reports showing that prenatal stress impairs spatial memory in radial arm maze and water maze tasks. To our knowledge, this is the first report of prenatal stress affecting memory for objects in novel spatial locations. Gestational stress-induced deficits in memory for spatial locations may serve as a useful model for studying psychopathologies with etiological links to the prenatal period.

THE β -CATENIN 'TARGETOME' IN LUNG: DYSREGULATION OF β -CATENIN UPSETS INFLAMMATORY BALANCE IN MOUSE AIRWAY SECRETORY CELLS

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β -catenin regulates cell function through transcriptional regulation of target genes and through secondary cascades that are modulated by the β -catenin target genes. We demonstrated that stabilization of β -catenin in mouse intrapulmonary Clara cells resulted in phenotypic alterations that were consistent with a block to post-natal maturation. However, β -catenin was unnecessary for this process. These two data sets supported the hypothesis that Clara cell immaturity in β -catenin stabilized mice was a consequence of alterations in secondary target gene expression. To address this hypothesis, gene expression was evaluated in groups of four 21-day old wild type, β -catenin stabilized, and β -catenin knockout mice using Affymetrix Mouse ST 1.0 gene arrays. Clusters of genes with altered expression patterns were identified using Ingenuity Pathway Analysis. Well-characterized β -catenin target genes were not differentially expressed in the stabilized group compared with wild type or knockout. Thus, persistent expression of β -catenin did not result in significant activation of direct target genes. In contrast, three cascades, ubiquitination, NF- κ B, and p38, were significantly changed in the stabilized group. These genes are not known to be direct targets of β -catenin, suggesting that dysregulation of β -catenin stability in Clara cells resulted in aberrant activation of secondary target genes. Based on these results, we conclude that stabilization of β -catenin blocks cellular maturation through inhibition of proteasome-mediated protein degradation and accumulation of pro-inflammatory proteins in these cells. These results suggest that mature Clara cells are critical regulators of inflammatory balance in the lung and have direct implications for

understanding disease initiation and progression in preterm birth.

NACL- INDUCED C-FOS EXPRESSION IN THE NUCLEUS OF THE SOLITARY TRACT OF MICE THAT LACK P2X RECEPTOR SUBUNITS NECESSARY FOR TASTE TRANSMISSION

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The gustatory nerves of mice that lack P2X2 and P2X3 receptor subunits (P2X dbl KO) are unresponsive to all taste stimulus qualities (Finger et al., 2005). Surprisingly, P2X dbl KO mice have residual behavioral responses to concentrated taste solutions, which may reflect non-gustatory or post-ingestive information presumably intact in P2X dbl KO mice. We previously measured brain activation in response to consumption of 150 mM monosodium glutamate (MSG), using the immediate early gene c-fos, in the nuc. of the solitary tract (nTS) - the primary central taste and viscerosensory nucleus. We found significantly less c-fos-like immunoreactivity (cFLI) in rostral (gustatory) levels of the nTS of P2X dbl KO animals as compared to WT controls. In contrast, cFLI did not differ between WT and P2X dbl KO mice in caudal (viscerosensory) nTS levels. However, MSG has a sodium component in addition to its primary glutamate component. Thus, the current study measured NaCl-induced c-fos activation. P2X dbl KO and WT mice were placed on 22 h water restriction 3 days prior to stimulation. On stimulation day, mice consumed water or 150 mM sodium chloride (NaCl) for 30 min. Following taste stimulation, mice were left undisturbed for approximately 60 min, perfused transcardially with buffered paraformaldehyde and then their brains were removed and processed for cFLI. For each genotype, the number of NaCl-induced c-fos-positive cells in the nTS was compared to the number induced by intake of water, yielding a measure of NaCl-dependent cell labeling. NaCl stimulation elicited little NaCl- dependent cFLI in either WT or P2X dbl KO animals, which did not differ between the two groups, and was not different from water- induced cFLI. Thus, MSG- induced nTS cFLI is attributable solely to the glutamate component of MSG.

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DEVELOPMENT OF AN INTELLECTUAL DISABILITIES GENE FUNCTION AND PATHWAY DATABASE

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Intellectual Disability (ID) affects some 2%-3% of the population of industrialized nations. Genetic causes of ID include (i) mutations in ~300 genes identified to cause ID as primary or secondary events, (ii) Down syndrome, trisomy of the long arm of chromosome 21 (HSA21q), and (iii) gene deletion and duplication in natural Copy Number Variation whose frequency and identity are only now being ascertained. We are developing the ID Gene Function and Pathway Database

(<https://gfuncpathdb.ucdenver.edu/iddrc/iddrc/home.php>) with the goals of: facilitating understanding of the genes which, when perturbed by mutation or gene dosage variation, cause ID; aiding in the description of pathways critical for ID; and identifying novel components of these pathways. Current contents of the database include three major categories:

(1) The ID database that is designed to provide integrated information on known and candidate ID genes, and their protein features, protein interactions and associated pathways. The goal is to aid both basic science and clinical researchers in new ID gene knowledge discovery and to facilitate hypothesis generation in the molecular basis of ID. The database is searchable by gene name, disease name, genomic location, among other features. The database currently contains a list of 357 genes retrieved from OMIM and the literature.

(2) The Human Chr21 gene database contains 508 genes identified by genomic sequence annotation of chromosome 21 and their conservation in the orthologous chimpanzee chromosome 21 and orthologous regions of mouse chromosomes 16, 17 and 10. These catalogues are searchable by gene name, gene functional class, open reading frame characteristics, conservation and genomic location.

(3) Chr21 SNP database contains a catalogue of SNP variation in RefSeq protein

coding genes of human chromosome 21; potential functional consequences of SNPs are described based on location with the mRNA and amino acid features of synonymous-nonsynonymous, conserved-nonconserved and location within a protein functional domain.

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p53 PREVENTS PROGRESSION OF NEVI TO MELANOMA PREDOMINANTLY THROUGH CELL CYCLE REGULATION

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In the United States, the incidence of malignant melanoma has increased faster than that of any other type of cancer in the last 50 years (477% increase from 1950 to 2000). This sharp increase in incidence highlights the necessity to uncover the underlying mechanisms of melanoma formation and to discover efficient therapeutic strategies. Conventional chemotherapy targets the p53 tumor suppressor pathway in many tumor types, however, melanocytes and melanoma cells are resistant to p53-dependent apoptosis. Thus, activation of p53 has been discounted as a potential therapeutic objective in this tumor type. On the other hand, evidence of p53 pathway disruption was found in melanoma, suggesting an important role in melanocyte transformation. Nevertheless, its mechanism of action is still unknown. To uncover when and how p53 is exerting its tumor suppressive activity, we followed the formation of pigmented lesions (nevi and melanoma) in a chemically treated *TP-ras*^{0/+} murine melanoma progression model with elevated p53 (*TP-ras*^{V12G} mice with deletion of one allele of *Mdm4*, the negative regulator of p53). The *TP-ras*^{0/+}; *Mdm4*^{+/-} mice developed fewer tumors with a delay in melanoma initiation with a dramatic decrease in tumor growth, lack of metastasis and an increase in survival compared to *TP-ras*^{0/+} mice. Thus, p53 effectively prevented the conversion of small benign tumors to malignant and metastatic melanoma. Chemical p53 activation in primary melanocytes and melanoma cell lines supported these findings. Global expression and network analysis of differentially regulated genes in melanocytes with activated p53 revealed the cell cycle regulator p21 as the top central node.

These data suggest that p53 plays an early role in melanoma prevention, prior to formation of a visible nevus, but it mainly acts on progression, limiting tumor growth and subsequent metastasis. Thus, specific activation of p53 in nevi on individuals who are

at high risk for melanoma may be a convenient strategy to mitigate some of this risk. Our findings are also in agreement with reports that in human melanomas, increased p53 levels predict a better prognosis and indicate that therapeutic targeting of p53 in melanoma needs to be revisited.

This work was funded by a Dermatology Foundation RCDA and an American Skin Association CDA.

CROSS REGULATION OF P53, AURORA KINASE-A AND MYC SIGNALING PATHWAYS IN MALIGNANT SKIN CARCINOMA

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We previously established a very highly malignant and metastatic murine cutaneous squamous cell carcinoma (SCC) model by co-expressing *Kras*^{G12D} and gain-of-function (GOF) *p53*^{R172H} mutant proteins. To determine how mutant p53 promotes malignant tumors, we performed expression profiling and comparative genomic hybridization (CGH) analysis on *p53*^{R172H/-} and *p53*^{-/-} tumors. Profiling and clustering experiments revealed four different stages of tumor progression, namely in ascending order of tumor progression, *p53*^{-/-} papillomas > *p53*^{R172H/-} papillomas > *p53*^{-/-} SCCs > *p53*^{R172H/-} SCCs. We characterized differentially regulated genes in the transition between papilloma to SCC using gene-ontology, network, or pathway analysis. SCC conversion in *p53*^{-/-} tumors revealed upregulation of mitosis/G2 checkpoint regulating genes and enrichment of chromosomal instability signatures. In contrast, SCC in *p53*^{R172H/-} tumors revealed enrichment of epithelial-to-mesenchymal transition and metastatic signatures. CGH analysis revealed more gains/losses in *p53*^{R172H} tumors than *p53*^{-/-} tumors, suggesting that genomic instability was acquired early in the evolution of *p53*^{R172H} tumors. Additionally, we observed amplification of *Aurora Kinase-A (AurKA)* or loss of its negative regulator, *AurKAIP* in 25% of *p53*^{R172H} SCC, indicating that deregulation of AurKA may mediate part of the genomic instability observed in GOF p53 tumors. Further analysis revealed that chromosome 15 was preferentially amplified in *p53*^{R172H/-} tumors. In particular, *Myc* on 15qD1 was highly amplified and correlated with increased protein levels of Myc, implicating Myc signaling in the metastatic nature of GOF p53 tumors. In summary, we hypothesize that GOF mutant p53 oncogenesis may be

mediated by the deregulation of both the Myc and AurKA signaling pathways.

PERSISTENT IMPAIRMENT OF REDOX STATUS DURING LITHIUM-PILOCARPINE-INDUCED EPILEPTOGENESIS

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Mitochondrial dysfunction and oxidative stress play a critical role in seizure-induced brain injury but their role during epileptogenesis remains unclear. Recent work in our laboratory (Jarrett et al., 2008) demonstrates increased hippocampal mitochondrial oxidative stress and DNA base excision repair of rats following kainate-induced (KA) status epilepticus (SE). To further establish the role of mitochondrial dysfunction in epileptogenesis, we determined indices of mitochondrial oxidative stress in a second model of temporal lobe epilepsy, the lithium-pilocarpine (Li-Pilo) model. Male Sprague-Dawley rats were injected with saline or pilocarpine hydrochloride (30 mg/kg) after treatment with lithium chloride (127 mg/kg) and scopolamine methyl bromide (1 mg/kg). Seizures were attenuated by injecting Diazepam (10 mg/kg) 90 min following SE and animals having spontaneous seizures were used in the study. The hippocampus, and mitochondria isolated from the hippocampus were obtained 24 h-3 mo following SE and used for HPLC analysis of glutathione (GSH), coenzyme A (CoASH), and hydrogen peroxide (H_2O_2) analyses by the Amplex Red assay. Genomic DNA was isolated from the hippocampus and piriform cortex and subject to QPCR analysis to measure oxidative lesion frequency. Mitochondrial H_2O_2 production and mtDNA lesion frequency increased in a time-dependent manner after SE peaking at 96 h, decreasing at 7 d during the so-called "latent period," and increasing again during the chronic phase following SE. Hippocampal GSH and CoASH were oxidized and depleted at all time points suggesting ongoing oxidative stress during epileptogenesis. Interestingly, a longer lag time in H_2O_2 production was observed in Li-Pilo-treated rats (~48-96h) compared to KA-treated rats (~24h) and we determined if this was related to the number of seizures experienced by rats in the two models. Li-Pilo-treated rats that experienced SE not attenuated by Diazepam showed an acute increase in H_2O_2 production and mtDNA lesion frequency

suggesting seizure-dependence of ROS production. These results demonstrate mitochondrial oxidative stress and genomic instability in two independent models of temporal lobe epilepsy, suggesting a role for mitochondrial dysfunction during epileptogenesis.

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ELEVATED EXPRESSION OF ERBB3 CONFERS PACLITAXEL RESISTANCE IN ERBB2-OVEREXPRESSING BREAST CANCER CELLS VIA UPREGULATION OF SURVIVIN

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Coexpression of erbB3 and erbB2 is frequently observed in breast cancer; and erbB3 plays a critical role for erbB2 promotion of breast cancer progression and anti-estrogen resistance. Here we determine the role of erbB3 in erbB2-mediated paclitaxel resistance in breast cancer cells. Overexpression of exogenous erbB3 via either stable or transient transfection in erbB2-overexpressing, but not erbB2-low, breast cancer cells significantly decreases paclitaxel-induced growth inhibition and apoptosis. Consistently, knockdown of erbB3 expression with a specific shRNA in breast cancer cells with coexpression of both erbB2 and erbB3 enhances paclitaxel-induced apoptosis evidenced by increased DNA fragmentation, PARP cleavage, and activation of caspase-3, and -8. Furthermore, while forced overexpression of erbB3 increases, specific knockdown of erbB3 decreases the expression levels of Survivin only in the erbB2-overexpressing breast cancer cells. Targeting Survivin with specific shRNA overcomes paclitaxel resistance without effect on the expression levels of either erbB2 or erbB3. Mechanistic studies indicate that the specific PI-3K inhibitor, but not the MEK inhibitor, not only abrogates erbB3-mediated upregulation of Survivin, but also reinforces the erbB2/erbB3-coexpressing breast cancer cells to paclitaxel-induced growth inhibition and apoptosis. Our studies demonstrate that heterodimerization of erbB2/erbB3 is pre-requisite for erbB2 tyrosine kinase activation; and elevated expression of erbB3 is required for erbB2-mediated paclitaxel resistance in breast cancer cells via PI-3K/Akt signaling-dependent upregulation of Survivin. These data suggest that novel strategies targeting erbB3 or Survivin may enhance the

efficacy of chemotherapeutic agents against erbB2-overexpressing breast cancer.

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DISRUPTION OF NORMAL TIGHT JUNCTIONAL CLAUDIN INTERACTIONS LEADS TO APOPTOSIS

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Background: Claudins, along with occludin, are integral cell-cell adhesion proteins that constitute the backbone of tight junction strands. Although the major role of claudins is thought to be to control paracellular diffusion, increasing evidence suggests that claudins may also play an important role in cell death signaling during pathogen infection and potentially during tumor progression. The goal of this study was to test the hypothesis that claudins are involved in initiating cell death signaling pathways when normal tight junctional binding is disrupted. Methods: A small peptide was designed that mimics a conserved sequence, DFYNP, in the second extracellular loop of claudin-3, -4, -7, and -8, the claudins most highly expressed in mouse and human mammary epithelial cells. A mammary epithelial cell line, EPH4, as well as primary mammary epithelial cells from wildtype and occludin knockout mice were treated with the mimic peptide. At various time points, cells were treated with fluorescent antibodies against claudin-4, occludin, and/or apoptotic makers (FADD, active caspase-8 and caspase-3) and fluorescence was imaged. Results: Peptide treatment induced mis-localization of claudin-4 from tight junctions to the cytosol. Within 16 hours of peptide exposure, EPH4 cells and primary mammary epithelial cells of wildtype mice became TUNEL positive and caspase-8 and -3 were activated, suggesting the induction of the extrinsic apoptotic pathway. Caspase activation was restricted to cells with disrupted, non-junctional claudin. Interestingly, disrupted claudin interactions also led to the mis-localization of occludin with non-junctional claudin-4 often co-localizing with non-junctional occludin within cytosolic vesicle-like structures. Within 4 hours, cytosolic claudin-4 and occludin co-localized with FADD and caspase-8, suggesting the formation of a Death Inducing Signaling Complex (DISC). Interestingly, the claudin mimic peptide did not induce apoptosis

in primary mammary epithelial cells of occludin knockout mice, suggesting that occludin is required for signaling of apoptosis when claudin binding is disrupted. Conclusions: Disruption of normal tight junctional claudin interactions leads to the mis-localization of claudin and occludin away from the tight junctions and initiates the assembly of the death inducing signaling complex, leading to apoptosis.

GENETIC SIGNATURES OF ACUTE MOUNTAIN SICKNESS.

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Acute mountain sickness (AMS) is the most common illness for sojourners to high altitude. Among people who travel from sea level to stay at moderate altitudes (1920-2960m), 27% exhibit AMS (Honigman 1993). Currently, there is no accurate way to predict who will get AMS among people never before exposed to high altitude. Additionally, the molecular mechanisms involved in the etiology or pathophysiology of AMS are relatively unknown. This study seeks to identify AMS gene expression hallmarks, both before ascent to simulated high altitude to discover candidate biomarkers for the prediction of AMS, and during the illness to more thoroughly describe AMS molecular characteristics. **METHODS:** In this study, 24 subjects were exposed to simulated high altitude (4878m) for ten hours using a hypobaric chamber. Peripheral blood mononuclear cells were collected from the subjects at baseline, one day prior to hypoxic exposure in ambient conditions (1600m; Aurora, CO), and after nine hours of hypoxic exposure. Genome-wide microarrays (Affymetrix HG-U133 Plus 2) measuring greater than 47,000 transcripts were used to profile gene expression changes with altitude exposure as well as gene expression in subjects who developed AMS compared with those who remained healthy. **RESULTS:** Exposure to hypoxia triggered the expression of genes in hypoxia-sensing pathways, as well as angiogenic and inflammatory pathways. Subjects with AMS compared with those who remained healthy showed increased expression of genes involved in hypoxia-sensing and brain

process pathways (eg ageing, effects of cocaine). Most interestingly, subjects who develop AMS show a unique pattern of gene expression in one hypoxia-related pathway, angiotensin II receptor, type I (AT1-R), differing from the pattern exhibited by subjects who remain healthy both before and during altitude exposure. Angiotensin has long been suspected to play a major role in AMS, but with limited, small-scale genetic analyses the relationship has not been confirmed.

CONCLUSIONS: Here, we show a relationship between AMS and the AT1-R pathway, with a possibility of using this pathway for developing a method for predicting risk of developing AMS prior to ascent to high altitude.

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HIGH RESOLUTION ANALYSIS OF COPY NUMBER VARIATION BREAKPOINTS REVEALS POTENTIALLY PREDISPOSING SEQUENCE MOTIFS AND VARIABLE MECHANISMS OF GENOMIC REARRANGEMENT

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Copy number variations (CNVs) are an increasingly recognized cause of human disease and variation. There is increasing evidence that unstable genome architecture promotes the rearrangements which lead to CNVs. This is especially true for CNVs that are associated with recurrent microdeletions and microduplications that lead to genomic disorders (GDs). In a majority of GDs the pathogenic CNVs are mediated by non-allelic homologous recombination (NAHR) between segmental duplications. In this study, we have identified and analyzed 100 pathogenic CNVs, 70 deletions and 30 duplications. The sizes of CNVs ranged from 10Kb to more than 56Mb. 17/100 (17%) CNVs resulted from NAHR between highly homologous segmental duplications, a significant enrichment, considering that SDs only make up 5% of the genome. Almost all of these 17 CNVs were found to be recurrent in multiple individuals and the frequency was directly proportional to the size and sequence identity shared between the paralogous SDs mediating NAHR. The remaining 83 CNVs were mostly non-recurrent, singletons with a few instances of overlapping CNVs but with different breakpoints. We used custom-designed, tiling microarrays to refine the rearrangement breakpoints in a subset of these allowing the rapid cloning and sequencing of 42 breakpoints. 25 breakpoints localized to repetitive DNA elements like Alus and LINES, but only three CNVs appeared to result from NAHR between repeats. The majority of the breakpoints appeared to result from Non-Homologous End Joining (NHEJ). Interestingly, for each of the CNVs, one to fourteen base pairs of microhomology was observed at the breakpoints further supporting the involvement of classic NHEJ and Ku-

independent NHEJ in the rearrangement. We further analyzed each of the breakpoint regions for sequence motifs and palindromic sequences that have previously been identified at or near rearrangement breakpoints. Although many such "hotspot" motifs were identified, none were significantly enriched when compared to control sequences. Analysis using MEME motif search program we identified a Poly(dA:dT) tract in the vicinity (average distance from BP is 85 bp) in 41/42 breakpoints. Poly(dA:dT) tracts have been shown to be important in nucleosome organization suggesting a correlation between breakpoints and chromatin structure. It will be interesting to see if these variable rearrangement mechanisms are observed as more CNV breakpoints, both pathogenic and normal variants, are analyzed.

STRUCTURE, MECHANISM, AND REGULATION OF A CRITICAL SPLICEOSOMAL ATPASE AND HELICASE BRR2

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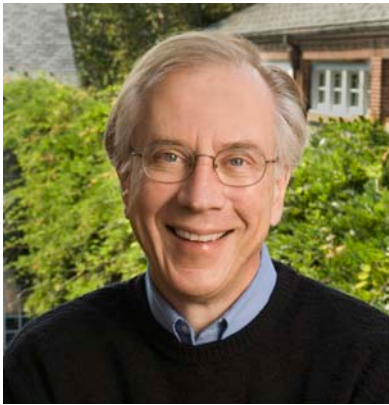
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Pre-mRNA splicing is an essential step in gene expression of all eukaryotes. Splicing of introns is carried out through two transesterification reactions catalyzed by the spliceosome, a huge RNA/protein complex composed of five snRNAs and over 100 protein factors. Structural and functional analyses are essential for our understanding of the molecular mechanism of pre-mRNA splicing. However, the structure and function of many spliceosomal components remain elusive. Among these are several DExD/H-box RNA helicases that play critical roles in the assembly and activation of the spliceosome. Brr2 is a large and essential helicase in the spliceosome. It is responsible for U4/U6 unwinding, a critical step in spliceosomal activation. Brr2 has a unique domain structure, which contains an N-terminal domain (NTD) and two tandem sets of a helicase domain followed by a Sec63 domain with unknown structure and function. To understand the structure and function of this critical spliceosomal helicase, we recently determined the crystal structure of the second Sec63 domain, which unexpectedly resembles domains 4 and 5 of DNA helicase Hel308. In addition, the helicase domain upstream of Sec63 has clear sequence similarity with domains 1-3 of Hel308. We, therefore, hypothesize that Brr2 is composed of an N-terminal domain and two consecutive Hel308-like modules, providing our first glimpse of the overall structure of this unique spliceosomal ATPase and helicase. The structural similarity between Brr2 and Hel308 suggests a helicase mechanism for Brr2 that is consistent with our mutagenesis and biochemical studies. This mechanism is different from many DEAD-box RNA helicases and is likely responsible for Brr2's unique ability to unwind the highly stable U4/U6 duplex. Furthermore, we demonstrated that the second Hel308 module of Brr2 interacts

with Prp8 and Snu114 in vitro and in vivo, potentially serving as a mediator for the regulation of Brr2's activity by Prp8 (an essential splicing factor known to stimulate Brr2's helicase activity). This is the first example of a helicase-like module serving as a major protein-interacting domain. We further demonstrated that the C-terminal region of Prp8 (Prp8-CTR) facilitates the binding of the Brr2/Prp8-CTR complex to U4/U6, suggesting a potential role of Prp8-CTR as an auxiliary substrate binding and specificity domain for Brr2. We are currently investigating the specific function of this domain using a combination of genetic, biochemical, and structural approaches. Our results in general have important implications for the mechanism and regulation of Brr2's activity.

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Dr. Thomas R. Cech

**Nobel Laureate in Chemistry, 1989
Distinguished Professor, UC-Boulder
Director, Colorado Initiative in Molecular
Biotechnology**

“How to Win a Nobel Prize”

Tom Cech was raised and educated in Iowa (B.A. in chemistry from Grinnell College, 1970). He obtained his Ph.D. in chemistry from the University of California, Berkeley, and then engaged in postdoctoral research in the department of biology at the Massachusetts Institute of Technology in Cambridge, Massachusetts. In 1978 he joined the faculty of the University of Colorado, Boulder, where he became a Howard Hughes Medical Institute investigator in 1988 and Distinguished Professor of Chemistry and Biochemistry in 1990. In 1982 Dr. Cech and his research group announced that an RNA molecule from *Tetrahymena*, a single-celled pond organism, cut and rejoined chemical bonds in the complete absence of proteins. Thus RNA was not restricted to being a passive carrier of genetic information, but could have an active role in cellular metabolism. This discovery of self-splicing RNA provided the first exception to the long-held belief that biological reactions are always catalyzed by proteins. In addition, it has been heralded as providing a new, plausible scenario for the origin of life; because RNA can be both an information-carrying molecule and a catalyst, perhaps the first self-reproducing system consisted of RNA alone. In January 2000, Dr. Cech moved to Maryland as president of the Howard Hughes Medical Institute, which is the nation's largest private biomedical research organization. In addition, HHMI has an \$80 million/year grants program that supports science education at all levels (K-12 through medical school) and international research. In April 2009, Dr. Cech returned to full-time research and teaching at the University of Colorado-Boulder, where he also directs the Colorado Initiative in Molecular Biotechnology.



Dr. Rodney Ulane

**NIH Training Officer and Director,
Division of Scientific Programs at OER
“NIH Grants for the Development of
Your Research Career:
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Process and How to Navigate It”**

Rod Ulane earned his PhD degree studying yeast genetics with Maurice Ogur at Southern Illinois University in 1971. He joined the NIH that year as a post doctoral fellow in the laboratory of Enrico Cabib in the former National Institute of Arthritis, Metabolism and Digestive Diseases, studying the developmental biology of yeast. In 1976, he joined the Child Health Institute as a senior staff fellow, and became a tenured staff scientist in that institute where he headed a laboratory investigating the developmental biology of the fetal lung. In 1979 he moved to the National Institute of General Medical Sciences where he was responsible for overseeing research training program reviews and program project reviews in the pharmacological sciences and structural biology, and biophysics. He left the NIH in 1990 to become associate professor of pharmacology and associate dean of the Graduate School of Biomedical Sciences, University of Texas Southwestern Graduate and Medical Schools in Dallas. At UT Southwestern he helped reorganize and consolidate PhD programs in the basic biomedical sciences, and served as co-director of the MD/PhD Program. Before coming back to the NIH in 2009, Dr. Ulane was Professor of Biomedical Education at the NYU School of Medicine, associate dean of biomedical research training, and director of the NYU MD/PhD program. Back at the NIH, Dr. Ulane works in the Office of the Director, overseeing NIH-wide research training policy.

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