

Second Annual Rhode Island Research Alliance Symposium

Program Book



Friday, October 2nd, 2009

RI Convention Center

One Sabin Street

Providence, RI

Program

- 7:30 a.m.** Poster set-up begins
- 8:00 a.m.** Registration and Continental Breakfast

Morning Session

- 9:00 a.m.** Welcome
- 9:15 a.m.** Robert K. Moyzis, Ph.D., University of California, Irvine
- 10:00 a.m.** **BREAK**
- 10:15 a.m.** WIH COBRE for Perinatal Biology
Carmen Marsit, Ph.D.
"Epigenetic Alterations as Markers of the Intrauterine Environment"
- 10:35 a.m.** RIH COBRE for Skeletal Health and Repair
Richard Terek, M.D.
"Anti-angiogenesis Strategies for Chondrosarcoma"
- 10:55 a.m.** RI EPSCoR, University of Rhode Island
Bethany Jenkins, Ph.D.
"Diatoms on a Diet: Profiling Genomes, Transcriptomes and Proteomes to Elucidate Responses to Limiting Nutrients"
- 11:15 a.m.** RI INBRE, University of Rhode Island
Niall Howlett, Ph.D.
"Maintaining Chromosome Stability: Role of the Fanconi Anemia-BRCA Pathway"
- 11:35 a.m.** RWMC COBRE for New Stem Cell Biology
Yinsheng Wan, Ph.D.
"The Molecular Mechanism of EGF-induced Cell Migration and Wound Healing"

Lunch and Poster Session

- 12:00 p.m.** Boxed Lunches in Rotunda and Poster Session in Ballrooms D/E
Leadership Luncheon (*invitation only*)

Afternoon Session

- 2:00 p.m.** Edward Seidel Ph.D., National Science Foundation
- 2:45 p.m.** RI INBRE, University of Rhode Island
Matthew Stoner, Ph.D.
“Mechanisms of Nuclear Receptor Signaling in Liver”
- 3:05 p.m.** Brown University
Ben J. Raphael, Ph.D.
“Structural Variation in Human and Cancer Genomes”
- 3:25 p.m.** **BREAK**
- 3:40 p.m.** Brown COBRE for Genetics and Genomics
Richard Freiman, Ph.D.
“Transcriptional Regulation of Ovarian Lifespan”
- 4:00 p.m.** RIH COBRE for Cancer Research Development
Rachel A. Altura, M.D.
“Survivin Signaling Networks in Cancer and Normal Development”
- 4:20 p.m.** RI INBRE, Salve Regina University
Bernard Munge, Ph.D.
“Nanomaterial-based Electrochemical Detection of Cancer Biomarker Proteins in Serum”
- 4:40 p.m.** Closing Remarks
- 5:00 p.m.** Reception

Abstracts for Rhode Island Speakers

Carmen J. Marsit, PhD

Assistant Professor of Medical Science, BioMed Pathology and Laboratory Medicine,
Brown University
COBRE for Perinatal Biology

“Epigenetic Alterations as Markers of the Intrauterine Environment”

The interaction between genes and the environment in which they are expressed is quickly becoming the focus of disease research in the post-genomic era. Sitting at this critical interface is the field of epigenetics, which provides mechanistic insight into the effect of the environment on gene expression, and which is proving to be vitally important in understanding the basis of human disease. The apparent plasticity of these epigenetic marks during development and their far-reaching effects throughout life make these alterations attractive in research focused on the developmental basis of adult disease, where the influence of the intrauterine environment is examined on pregnancy and perinatal outcomes as well as outcomes throughout the life course. Sitting at the interface of the environment and the developing fetus is the placenta, playing a role not only in nutrient transport, but also in hormonal regulation and in protection of the fetus from an array of insults. These varied and vital roles are reflected in the highly orchestrated cell biology and gene expression patterns of the major placental cells, the cytotrophoblasts, which must be able to react appropriately to a variety of stimuli and insults to perform their function. Thus, we have undertaken a multi-faceted approach to examining the epigenetic landscape of the human placenta comparing the growth-restricted to non-restricted intrauterine environments. To this end, we have obtained a cohort of greater than 400 infants with appropriately characterized and preserved placental tissue. Our examinations are focusing on the epigenetic mechanisms of DNA methylation and miRNA expression. We have begun examination of the overall global methylation status of these samples utilizing quantitative pyrosequencing technologies, and will be examining gene-specific DNA methylation profiles utilizing genome-wide array-based approaches as well as targeted, candidate gene examinations. In addition, we have examined the expression profile of miRNA in the placenta and have identified differential profiles of miRNA expression between growth restricted and non-restricted samples, results which we are currently validating in a larger, independent series of samples. These experiments are serving as a basis for future work to describe in larger, population-based cohorts, the etiology and clinical significance of epigenetic alterations in perinatal biology.

Richard Terek

Associate Professor, Department of Orthopaedic Surgery, Warren Alpert Medical School
of Brown University

“Anti-angiogenesis Strategies for Chondrosarcoma”

Chondrosarcoma, along with osteosarcoma and Ewing sarcoma, comprise the three most common primary bone cancers. However, unlike osteosarcoma and Ewing sarcoma, the cure rate for chondrosarcoma, which ranges from 10-25%, has not improved over the last several decades owing to its lack of response to cytotoxic chemotherapy. The majority of patients succumb to lung metastases. In contrast to cytotoxic chemotherapy, biologic based, targeted therapy attempts to counteract the molecular abnormalities that drive tumor growth and metastasis. Critical to both of these processes is angiogenesis, which represents the ability of a tumor to induce new blood vessel growth from surrounding normal tissue. One strategy for inhibiting tumor growth and metastasis is to inhibit tumor induced angiogenesis. Our work has focused on understanding the molecular mechanisms underlying angiogenesis in chondrosarcoma.

Angiogenesis in chondrosarcoma is induced by hypoxia, re-expression of developmental pathways, and genetic aberrations. As in many other cancers, angiogenesis is largely driven by the cytokine vascularized endothelial growth factor (VEGF). Although chondrosarcoma is phenotypically related to cartilage, an avascular, hypoxic tissue, hypoxia induced expression of hypoxia inducing factor one alpha (HIF-1a) and its regulation of downstream targets including VEGF are intact. In addition, we have shown that a developmental pathway regulating growth plate maturation culminating in endochondral ossification and angiogenesis that involves histone deacetylase 4, the transcription factor RUNX2, and VEGF, is recapitulated in chondrosarcoma. Aberrant expression of chemokine receptor four (CXCR4) is also present in chondrosarcoma and there is cross talk with hypoxia mediated pathways.

CXCR4 and its ligand, stromal derived factor 1 (SDF1) promotes tumor metastasis by mediating proliferation and migration of tumor cells and enhancing tumor-associated angiogenesis. CXCR4 and SDF1 are upregulated in primary chondrosarcoma tumors compared to normal articular cartilage, and CXCR4 is upregulated in chondrosarcoma cell line JJ compared to normal chondrocytes. Hypoxia and specifically HIF-1a further increase CXCR4, VEGF, and matrix metalloproteinase 1 (MMP1) expression and invasion in chondrosarcoma cells in vitro, and conditioned media from these cells stimulates angiogenesis in vitro. All of these findings are inhibited by siRNA directed at HIF-1a or CXCR4, the CXCR4 inhibitor AMD3100, as well as with ERK inhibitor U0126. CXCR4 expression is further increased when JJ cells are grown in a xenograft mouse model compared to hypoxic culture. Since directly counteracting the effects of hypoxia on the aggressive phenotype of cancer cells is not possible, whereas CXCR4 blocking agents approved for human use are available, we will test the effect of CXCR4 blockade on

chondrosarcoma angiogenesis and metastasis in a xenograft mouse model using bioimaging, direct measurement of tumor growth, and survival analysis.

Bethany Jenkins, PhD

Assistant Professor, Department Of Cell and Molecular Biology and Graduate School of Oceanography, University of Rhode Island

“Diatoms on a Diet: Profiling Genomes, Transcriptomes and Proteomes to Elucidate Responses to Limiting Nutrients”

Diatoms are unicellular photosynthetic algae that fix approximately 20% of all carbon on earth. Nutrient availability constrains the efficiency of diatom growth and primary production. Many ocean environments are limited for one or more nutrients and this limitation constrains carbon fixation on basin-wide scales. In addition, as oceanic temperature and CO₂ levels rise in response to anthropogenic climate change, nutrient cycling and diatom production will be impacted. My laboratory has been using a combination of physiological experiments, quantitative gene expression analysis, and genome-wide transcriptome and proteome profiling to better understand nutrient physiology in diatoms.

Iron is an important element for photosynthesis and uptake of other essential nutrients such as nitrate. Diatoms from open ocean ecosystems often experience chronic iron limitation, whereas coastal species grow in much higher iron concentrations. We have compared the response of coastal and oceanic diatoms to iron stress and followed genes implicated in lowering iron demand and in iron uptake. Results from quantitative PCR analysis show coastal and oceanic diatoms regulate these genes differently in response to iron limitation. Working with colleagues at URI and the Woods Hole Oceanographic Institute, we are profiling the genome-wide responses of the model diatom, *Thalassiosira pseudonana*, for which a complete genome sequence is available, to nutrient limitation. Results from a phosphorus limitation experiment show the up-regulation of phosphorus-related genes, including alkaline phosphatases and phosphate transporters, in the global transcriptomic and proteomic analysis. Experiments are underway to profile iron metabolism and iron and phosphorus co-limitation. In collaboration with the Joint Genome Institute, we are conducting similar experiments with the diatom *Thalassiosira rotula*, a more abundant species, but one that has not been sequenced. The experiments with *T. rotula* facilitate the development of new methodologies for working with non-model organisms. Data from experiments with multiple environmental conditions and species will allow us to compare how different species regulate their nutrient metabolism and how they respond to environmental stressors.

Niall G. Howlett, Ph.D.

Assistant Professor, Department of Cell and Molecular Biology
University of Rhode Island
RI-INBRE

“Maintaining Chromosome Stability: Role of the Fanconi Anemia-BRCA Pathway”

Fanconi anemia (FA) is a rare recessive disease characterized by congenital abnormalities, progressive bone marrow failure, and pronounced cancer susceptibility. The FA proteins, and the protein products of the major breast cancer susceptibility genes *BRCA1* and *FANCD1/BRCA2* co-operate in a common pathway, the FA-BRCA pathway, to repair damaged DNA and to prevent cellular transformation. A critical step in the activation of the FA-BRCA pathway is the mono-ubiquitination of the FANCD2 and FANCI proteins, which occurs following exposure to DNA damaging agents and during S phase of the cell cycle. FANCD2 and FANCI mono-ubiquitination signals their translocation to discrete nuclear foci where they co-localize with several well known DNA repair proteins including BRCA1, FANCD1/BRCA2, and RAD51, strongly suggestive of an important role for this pathway in homologous recombination DNA repair. Importantly, the regulation of the mono-ubiquitination of FANCD2 (*and FANCI*), as well as its precise function in DNA repair, remain poorly understood. Using bioinformatics approaches we have recently determined that the FANCD2 protein harbors two putative PCNA-interaction motifs (PIP-box), as well as a putative ubiquitin-binding and ubiquitin-like domain. We hypothesize that these domains play critical roles in the regulation of the mono-ubiquitination of FANCD2. To begin to test this hypothesis, using a site-directed mutagenesis approach we have mutated the key residues of one of the FANCD2 PIP-boxes: We have determined that the disruption of this motif abrogates FANCD2-PCNA binding and precludes both spontaneous and DNA damage-inducible FANCD2 mono-ubiquitination. Extending upon these findings we have recently established that the PCNA regulatory protein p21^{Cip1/Waf1} is also required for efficient DNA damage-inducible FANCD2 mono-ubiquitination: a human colorectal carcinoma cell line harboring a targeted homozygous deletion at the *p21* locus fails to up-regulate the mono-ubiquitination of FANCD2 following exposure to DNA damaging agents. Furthermore, like FANCD2 and PCNA, FANCD2 and p21 physically interact. Our results suggest that the PCNA and p21 proteins coordinately regulate the activation of the mono-ubiquitination of FANCD2 during the cellular DNA damage response, and thus provide novel insight into the regulation of this important tumor suppressor pathway. A greater understanding of the regulation of the mono-ubiquitination of the FANCD2 protein may provide important clues as to the origins of the congenital abnormalities, bone marrow failure and cancer susceptibility characteristic of FA patients, and improve diagnostic and therapeutic approaches to FA.

Yinsheng Wan, Ph.D.

Associate Professor of Department of Biology
Providence College

COBRE Executive Committee and Member of INBRE (2009-2010)

“The molecular mechanism of EGF-induced cell migration and wound healing”

Cell migration is one of the major events toward wound healing. Epidermal growth factor or EGF has been widely applied to clinically manage the wound healing process. However, the cellular and molecular mechanism of EGF-induced cell migration towards wound healing is far from clear. We have shown in our previous studies that EGFR mediates EGF-induced cell migration via MAPK/AKT pathway leading to AQP3 upregulation. Our most recent studies have revealed unexpected but very exciting results that Gi proteins play critical roles in EGF-induced MAPK and AKT/mTOR signaling, and Gi1/3 double knockout abolishes EGF-induced AKT/mTOR/ERK MAPK activation. These Gi proteins associate with Gab1, a key adaptor protein for EGFR signal, and the interaction between Gi proteins and Gab1 is essential for Gab1 function. Gi protein deficiency abolishes Gab1 binding to EGFR and Gab1 phosphorylation or function as an adaptor proteins. Gi1/3 double siRNA also largely impairs EGF-induced AKT/mTOR/MAPK activation. Furthermore, Gi protein vectors can rescue MAPK/AKT/mTOR signals induced by EGF. Given the critical roles of Gi proteins in EGF-induced signal transduction, and EGFR/MAPK/AKT is important for AQPs up-regulation mediating cell migration and wound healing, we most recently hypothesized that Gi proteins play important roles in EGF-induced cell migration and wound healing, probably by mediating AKT/mTOR/MAPK signaling and AQPs up-regulation. We are currently investigating the possible roles of Gi proteins in EGF-induced cell migration and wound healing, testing the roles of mTORC1 and mTORC2 in EGF-induced cell migration and wound healing, and investigating whether Gi proteins and mTORC1 and mTORC2 components contribute to EGF-induced AQPs up-regulation that leads to cell migration and wound healing. We expect to provide more insights into the molecular mechanism of EGF-induced cell migration and wound healing.

Matthew Stoner, PhD

Research Assistant Professor, Department of Biomedical & Pharmaceutical Sciences and
Rhode Island IDeA Network of Biomedical Research Excellence
University of Rhode Island

“Mechanisms of Nuclear Receptor Signaling in Liver”

In the liver, both endogenous and exogenous chemicals are metabolized by a series of enzymatic steps to yield secondary products that are more easily excreted and usually less toxic than their parent compounds. Hepatocytes are specialized liver cells that make up approximately 80% of the total mass of the organ and they express the majority of drug metabolizing enzymes and are rich in various nuclear receptor proteins that are the molecular sensors of toxic insult. Constitutive androstane receptor (CAR) is a unique transcription factor that is expressed in hepatocytes and multiple structurally-diverse chemicals modulate CAR-target gene expression. Additionally, CAR-dependent gene regulation varies among individuals due to differences in expression of CAR at the level of mRNA splicing and translation of proteins from alternate start sites. Transcriptional activation and deactivation of CAR occurs partly through well-defined ligand-dependent binding; however, CAR activity is regulated also through a more complex indirect and ligand-independent signaling pathway. Studies are designed to explain the mechanisms of CAR activation in order to gain a better understanding of nuclear receptor regulation in liver; one application of this knowledge is the refined prediction of potential adverse drug-drug interactions.

(Supported by RI-INBRE Grant # P20RR016457 from NCRR/NIH).

Ben Raphael

Department of Computer Science & Center for Computational Molecular Biology
Brown University

“Structural Variation in Human and Cancer Genomes”

Structural variants -- including duplications, insertions, deletions, and inversions of large blocks of DNA sequence -- are an important contributor to natural human genome variation and also frequently occur in cancer genomes. I will describe computational techniques that we have developed for analysis of structural variants. These include: (i) methods to classify and compare variants measured in different individuals using a variety of DNA sequencing technologies; (ii) combinatorial algorithms to analyze overlapping genome rearrangements and duplications observed in both normal and cancer genomes. I will illustrate the applications of our techniques to data from the 1000 Genomes Project and The Cancer Genome Atlas.

Richard N. Freiman, Ph.D.

Assistant Professor of Medical Science, Molecular and Cell Biology and Biochemistry,
Brown University, COBRE for Genetics and Genomics

“Transcriptional Regulation of Ovarian Lifespan”

The mammalian ovary is unique in that its reproductive lifespan is limited by oocyte quantity and quality. Oocytes are recruited from a finite pool of primordial follicles that are usually exhausted from the ovary during mid-adult life. If regulation of this pool is perturbed, the reproductive capacity of the ovary is compromised. TAF4b is a gonadal-enriched subunit of the TFIID complex required for female fertility in mice. Previous characterization of TAF4b-deficient ovaries revealed several reproductive deficits that collectively result in infertility. However, the etiology of such fertility defects remains unknown. By assaying estrous cyclicity, ovarian pathology and gene expression changes in young TAF4b-deficient female mice, we hypothesize that TAF4b-deficient females exhibit premature reproductive senescence. The rapid decline of ovarian function in TAF4b-null mice begins in early postnatal life and follicle depletion is completed by sixteen weeks. To uncover differences in gene expression that may underlie accelerated ovarian aging, we compared genome-wide expression profiles of three week old, pre-pubescent TAF4b-null and wild-type ovaries. At three weeks of age, decreased gene expression in TAF4b-null ovaries is similar to those seen in aged ovaries revealing several molecular signatures of premature reproductive senescence. One significantly reduced transcript in the young TAF4b-null ovary encodes Mov10-L1, a germline-specific RNA helicase that is related to the Drosophila RNA interference protein Armitage. In addition, the meiosis-specific cohesin subunit Smc1b is reduced in the immature TAF4b-deficient ovary suggesting that meiotic defects may underlie the premature depletion of ovarian follicles in the TAF4b-deficient mice. We conclude that rather than be involved in the establishment of the primordial follicle pool during embryogenesis, TAF4b regulates gene expression programs that are required to properly maintain the quality of oocytes and their cognate primordial follicles that helps establish the appropriate female reproductive lifespan.

Rachel Altura

Associate Professor of Pediatrics
Rhode Island Hospital and the Warren Alpert Medical School at Brown University

“Survivin Signaling Networks in Cancer and Normal Development”

An estimated 1 in 4 people die of cancer each year, making this disease one of the major threats to global human health. To improve survival rates, we must discover novel agents that target molecular pathways unique to cancer cells. Ideally, these pathways should involve nodal proteins that are exclusively expressed in cancer cells but deficient

in normal healthy tissue. Survivin is a cancer-associated protein with dual roles as an inhibitor of cell death and as a regulator of cell division. It is expressed at high levels in all malignant cancer cells, with especially elevated levels in cancers that are resistant to standard treatments, making it a prognostic indicator for high risk disease. By contrast, its expression is diminished in healthy, differentiated tissues making it an ideal candidate for directed cancer therapy. The focus of our laboratory is to examine the role of survivin in both normal developmental and cancer cell pathways, with the goal to identify differential functions for this protein among these different cell types. Recently, we discovered a novel regulatory mechanism for survivin in tumor cells. This mechanism appears to be linked to its nuclear function within these cells. Targeting this regulatory mechanism may represent a novel and potentially improved methodology for biologic therapy.

Bernard S. Munge, Ph.D.

Assistant Professor of Chemistry, Department of Chemistry,
Salve Regina University
INBRE

“Nanomaterial-based Electrochemical Detection of Cancer Biomarker Proteins in Serum”

Despite recent advances in treatment, cancer still remains a major leading cause of death in the world. Rapid, specific *early detection* of cancer biomarkers proteins in serum coupled with new therapies is the only hope to change this fact. Such sensitive detection schemes are expected to greatly improve patient prognoses, treatment success, and even lead to cancer prevention. The broad long-term goals are to develop nanomaterial-based arrays to measure collections of early cancer biomarker proteins for specific forms of cancer. We have developed sensitive electrochemical immunosensors for the detection of various cancer biomarker proteins in clinically relevant calf serum samples. Such sensor devices feature vertically aligned single wall carbon nanotubes (SWNT) arrays and/or gold decorated electrode platforms with capture antibodies. The biomarker protein detection is based on a sandwich immunosensor captured on either SWNT or glutathione protected gold nanoparticles (GSH-AuNP) coupled with catalytic reactions involving horseradish peroxidase tracer secondary antibody. Several signal amplification strategies have been developed to enhance the sensitivity and detection limits including carbon nanotube (CNT), polymeric beads and magnetic beads multi-label secondary antibody bioconjugates which maximizes the number of enzyme labels per binding event. Greatly amplified sensitivity for SWNT immunosensor was attained by using bioconjugates featuring horseradish peroxidase (HRP) labels and secondary antibodies (Ab₂) linked to carbon nanotubes (CNT) at high HRP/Ab₂ ratio. This approach provided a detection limit of 4 pg mL⁻¹ (100 amol mL⁻¹), for prostate specific antigen (PSA) in 10 μL of undiluted calf serum, a mass detection limit of 40 fg. Accurate

detection of PSA in human serum samples was demonstrated by comparison to standard ELISA assays. PSA was quantitatively measured in prostate tissue samples for which PSA could not be differentiated by the gold standard immunohistochemical staining method. Similar results were obtained for MMP-3 using polymer beads amplification strategy. We also recently compared the sensitivity and performance of our SWNT immunosensor to the GSH-AuNP platform using IL-6 cancer biomarker protein under the same experimental conditions. The GSH-AuNP immunosensor gave a detection limit (DL) of 10 pg mL^{-1} IL-6 (500 amol mL^{-1}) in $10 \text{ }\mu\text{L}$ calf serum, which was 3-fold better than 30 pg mL^{-1} found for the SWNT immunosensor for the same assay protocol. We are currently exploring multi-labeled particles with thousands of HRPs per Ab_2 along with AuNP platforms to further enhance sensitivity and detection limit. Both the SWNT and GSH-AuNP immunosensors can be adapted easily for the detection of other relevant biomarkers and have the potential for fabrication into arrays to facilitate multiplexed detection. We believe that such devices will evolve toward a very promising future for reliable point-of-care diagnostics of cancer and other diseases, and as tools for intra-operation pathological testing, proteomics, and systems biology.

Abstracts of Poster Presentations

Almeida, Karen H. – Rhode Island College	15
Ardito, Matthew – EpiVax, Inc	16
Berrios-Candelaria, Rosalie – Rhode Island College	17
Brown, Breann L. – Brown University	18
Buhlmann, Janet – EpiVax, Inc.	19
Byrd, Angel S. – Rhode Island Hospital	20
Cao, Cong – Brown University	21
Clifton, James – Brown University	22
Cohen, Toby – EpiVax, Inc.	23
Cook, Richard B. – BioScience Bead Division of CSS, Inc.	24
Copp, Laura – EpiVax, Inc.	25
Cousens, Leslie P. – Roger Williams Medical Center	26
Davidson, Shawn – Providence College	27
Desjarlais, Jennifer – Rhode Island College	28
Desroches, Bethany – Rhode Island Hospital	29
Dupont, Rachel – EpiVax, Inc.	30
Flight, Patrick A. – Brown University	31
Gervasi, Carissa – Roger Williams University	32
Hammerman, Scott – Roger Williams Medical Center	33
Howlett, Niall G. – University of Rhode Island	34

Huang, Helen (He) – University of Rhode Island	35, 36
Jayasuriya, Chathuraka – Brown University	37
King, Michelle – Rhode Island Hospital	38
Klysik, Jan – Brown University	39
Lai, Zhongbin – Women & Infant’s Hospital	40
LaLiberte, Alyse – Women & Infant’s Hospital	41
Lin, Xiaofeng – Roger Williams Medical Center	42
Linden, Jennifer – Brown University	43
Linehan, Jennifer – Roger Williams University	44
Lucht, Brett – University of Rhode Island	45
Luks, Valerie L. – Roger Williams Medical Center	46
Luo, Luguang – Roger Williams Hospital	47, 48, 49
Maccani, Matthew A. – Brown University	50
Massari, Todd – Roger Williams University	51
Meitner, Trish – Rhode Island Hospital	52
Moise, Lenny – EpiVax, Inc	53
Murphy, Kevin R. – Providence College	54
Nevers, Tania – Women & Infants Hospital	55
Newsome, Courtni T. – Brown University/RIH	56
Norris, Wendy – Women & Infants Hospital	57
Pagidas, Kelly – Women & Infants Hospital	58
Sadowska, Grazyna - Women & Infants Hospital	59

Sanders, Jennifer – Rhode Island Hospital	60
Selliah, Nithianandan – Roger Williams Medical Center	61
Shakarian, Alison M. – Salve Regina University	62
Sharma, Meenakshi – University of Rhode Island	63
Solares, Jose – Rhode Island College	64
Spasova, Mariya - Women & Infants Hospital	65
Stoner, Matthew – University of Rhode Island	66
Symington, Steven B. – Salve Regina University	67
Tarquino, Keiko – Rhode Island Hospital	68
Tassone, Ryan – EpiVax, Inc.	69
Terry, Frances E. – Roger Williams Medical Center	70
Thanawala, Vaidehi – University of Rhode Island	71
Tseng, Yi-Tang – Women & Infant’s Hospital	72
VanDijk, Greg – EpiVax, Inc.	73
Vecchione, James J. & Jason K. Sello – Brown University	74
Wang, Xiao Tian – Roger Williams Medical Center	75, 76
Weber, Constanze – EpiVax, Inc.	77
Webster, Thomas J. – Brown University	78
Yakirevich, Evgeny – Rhode Island Hospital	79
Yano, Naohiro – Women & Infants Hospital	80
Zhou, Fiona – Rhode Island Hospital	81

Bloom Syndrome Protein: A Study of Protein Partnerships

K.L. Bergeron, E.L. Murphy and K.H. Almeida

Rhode Island College, Providence, RI

Problem: Bloom syndrome (BS) is a rare recessive disorder characterized by premature death due to a predisposition to a wide array of clinical symptoms including photosensitivity, immunodeficiency and cancer. An increase in genomic instability is used to diagnose Bloom Syndrome however there is no known cure. BS is caused by mutations of the BLM gene. Specific regions of the BLM protein may affect genomic stability by partnering with key members of the Homologous Recombination (HR) DNA repair pathway. Our lab focuses on the protein partnerships of BLM protein to determine their influence on HR.

Method: A systematic set of deletion mutants has been generated for each terminus of BLM. DNA corresponding to each polypeptide fragment was cloned into Gateway entry vectors, sequenced and recombined for expression in *E. coli*. Protein partnerships with specific BLM regions were defined by far western immunoblot analysis and confirmed that the N-terminus and C-terminus of BLM interact with Rad51, the central protein in the HR pathway.

Results: Specifically, the last 114 amino acids of the N-terminus (BLM₁₀₀₋₂₁₄) and the first 50 amino acids of the C-terminus (BLM₁₃₁₇₋₁₃₆₇) are responsible for the interaction with Rad51. The majority of the interaction on the N-terminus is housed between amino acids 150-214 (55%), with BLM₁₀₀₋₁₅₀ and BLM₁₃₁₇₋₁₃₆₇ contributing 21% and 24% respectively. These studies will clarify the role of BLM in maintaining genomic stability.

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ROLE OF TREGS: INSIGHT FOR THE DESIGN OF AN IMPROVED HEPATITIS C VACCINE

Shuo Li, *Stefan Floess, **Gaudieri Silvana, **Andrew Lucas, *Alf Hamann, ***Matt Ardito, ***Anne S. De Groot, ***William Martin, and Eric Gowans
Macfarlane Burnet Institute for Medical Research and Public Health, Melbourne, Australia; *Experimental Rheumatology, Charite University Medicine, Berlin, Germany; **Centre for Clinical Immunology and Biomedical Statistics, Perth, Western Australia; ***EpiVax, Inc. Providence, RI USA

Problem: Hepatitis C is a small positive sense single stranded RNA virus that causes persistent infection that leads to liver failure. CD4+ and CD8+ T cell responses decrease as HCV progresses from acute to persistent infection. Recent studies suggest that HCV progression is associated with functional changes in regulatory T cells. A proportion of natural CD25+ cells isolated from the PBMC of HCV patients can substantially up-regulate CD25 expression in response to HCV peptide stimulation in vitro, thus HCV-specific Tregs may be primed and expanded during the disease.

Methods: Expression profiles (cell death, activation, proliferation and transcriptional regulation) suggest a survival advantage of HCV-responsive Tregs. An epigenetic analysis of the FOXP3 locus in HCV-responsive Treg shows that this locus is completely demethylated. We also performed in vitro studies of T cell function and phenotype. Each of the HCV peptides was mapped using EpiMatrix for the presence of an HLA binding motifs.

Results: CD25 expression on FOXP3+ cells were shown to be up-regulated by the HCV antigen. The FOXP3 expressing cells are stable Tregs and the responsiveness likely reflect their TCR specificity to HCV antigens. The positive correlation of in vitro Treg response with in silico EpiMatrix predictions provides strong evidence for the existence of dominant promiscuous Treg epitopes.

Conclusions: HCV-responsive natural CD25+ cells are not activated conventional T cells expressing FOXP3, but hard-wired Treg with a stable FOXP3 phenotype and function. Information on the identity and location of Treg epitopes in HCV proteins will facilitate novel HCV vaccine design.

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Differentiation of Out-Group Members: Relative Intergroup Status and Familiarity

Rosalie Berrios-Candelaria, Tiia Nurmiko, Peter Murphy, Lorin Kinney,
Beth Lewis, Thomas E. Malloy
Department of Psychology, Rhode Island College

Problem: The group one is a member of is the in-group; groups that one is not a member of are out-groups. Social psychological research shows that individuals fail to differentiate the unique traits of out-group members, while they do differentiate the unique traits of in-group members. This is termed the out-group homogeneity bias; a perceiver concludes “they’re all the same” when judging out-group members. Is this bias due to relative group status, a lack of familiarity with the out-group, or both?

Methods: Using a minimal group paradigm involving a dot estimation procedure, we manipulated familiarity with the out-group and the relative status of the in-group and out-group in a 2 x 2 design. Traits of out-group members were rated; data were analyzed using variance component analysis.

Results: A significant main effect due to relative group status was observed ($F(29,29) = 3.76, p < .01$); perceivers from a low status group differentiated the traits of high status out-group members to a greater extent than did high status perceivers when judging low status out-group members. A significant main effect for familiarity was found but was not predicted ($F(29,29) = 2.15, p < .05$). There was less differentiation of out-group members in a high familiarity condition compared to a low familiarity condition.

Conclusions: Out-group homogeneity is due, in part, to differential group status. Members of a high status group focus on the features that define category membership (e.g. a foreign accent) and ignore features that permit differentiation of category members.

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Institution: Rhode Island College

Three dimensional structure of the MqsR:MqsA complex: A novel toxin:antitoxin pair regulating bacterial persistence

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Bacterial biofilms are communities of bacterial cells that typically adhere to a variety of surfaces and are present in over 85% of bacterial infections. One mechanism by which biofilms evade antibiotic challenge is through the presence of persister cells. Persister cells comprise approximately 1% of a biofilm, and because they are dormant, persisters are resistant to antibiotics. Moreover, after removal of antibiotic, persisters are capable of reverting to the normal growth state and regenerating the biofilm population. Persister cells are known to upregulate a class of proteins known as toxin-antitoxin (TA) modules which are characterized by a stable toxin and a highly labile antitoxin. Several TA modules across many bacterial species have been identified. Under normal conditions, the toxin and antitoxin form a tight complex that sequesters the toxin away from its cellular targets. However, under conditions of environmental stress or antibiotic challenge, the labile antitoxin is rapidly degraded which enables the free toxin to inhibit cell growth. Interestingly, the toxin that is most differentially upregulated in persister cells is MqsR. Here we report the structure and function of unbound MqsA, the antitoxin partner of the MqsR toxin, as well as the MqsR:MqsA N-terminal domain complex. MqsA is a homodimer consisting of two N-terminal zinc binding domains characterized by a novel MqsA fold while the two C-terminal domains exhibit a XRE-HTH (helix-turn-helix) fold and mediate binding to the *mqsRA* promoter. MqsR is structurally homologous to the toxin RelE and is predicted to function as a ribosome-dependent ribonuclease.

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**IGG TREGITOPES AND AITD-ASATI:
ANTIGEN SPECIFIC TOLERANCE INDUCTION IN AUTOIMMUNE THYROID DISEASE**

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Problem: We hypothesized the expansion of natural regulatory cells in response to Tregitopes would restore the balance between autoimmune effectors and regulatory cells, thus suppressing anti-TSH-R responses associated with autoimmune thyroid disease.

Methods: PBMC from patients with GD were cultured +/- Tregitopes for 4 days, then analyzed for the presence of regulatory T cells. PBMCs were cultured with GD antigens +/- T regitopes, then IFN γ production was evaluated by ELISpot. HLA DR2 mice were immunized repeatedly with TSH-R in CFA +/- Tregitopes. On day 21, mice were sacrificed and the proliferative response to GD peptide stimulation was assessed.

Results: Co-incubation of PBMC with GD antigens and hTregitopes leads to a significant suppression of the immune response to the immunogenic epitopes in both human (one subject) and DR 1501 mice (two separate experiments). In separate experiments we demonstrated incubation of human PBMC leads to the expansion of CD4/CD25hi/FoxP3 subset and to the induction of IL 10 secretion. We have also shown bystander suppression of immune responses to dust mite lysate and other common antigens (Flu HA, Tet Tox, CEF) in vitro.

Conclusion: The data obtained to date support our hypothesis that Tregitopes can be used to induce antigen-specific adaptive tolerance. Our pilot studies using TSH-R epitopes (in vitro) and TSH-R ECD (in vivo) indicate that this may also be possible in the context of Graves' Disease. This research may lead to the use of Tregitopes as a tolerance-inducing treatment in early-onset Graves' Disease and possibly also in Hashimoto's thyroiditis.

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β_2 INTEGRIN COMPLEMENT RECEPTOR 3 (CR3) (CD11b/CD18) REGULATION OF NEUTROPHIL FUNCTION

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Problem: Complement Receptor 3 (CR3) is a β_2 integrin that is unique because it contains a polysaccharide binding lectin-like domain (LLD), in addition to the conventional binding domain (I-domain). A number of neutrophil functions have been shown to be altered, including adhesion, diapedesis and cytotoxicity, depending on single or dual ligation of CR3. Our goals are to analyze downstream signaling pathways once the I-domain alone is bound; once the LLD alone is bound; once both domains are bound. We hypothesize that single or dual ligation of CR3 will result in stimulation of different intracellular pathways, determining the character of the cell.

Methods: Experiments were done using neutrophils isolated from human blood. To identify several components of CR3 downstream signaling we have immunoprecipitated molecules that are tyrosine phosphorylated in response to immobilized fibrinogen, fibronectin and (β) (1-3) D-glucan +/- tumor necrosis factor alpha (TNF α) or formylmethionylleucylphenylalanine (fMLP). In addition, Cellomic analysis and observational studies provided changes in morphology and tyrosine phosphorylation of adhered neutrophils stimulated with antibodies or ligands.

Results: These various studies elucidated downstream signaling molecules once the I-domain alone is bound; once the LLD alone is bound; once both domains are bound. Once the domain specific stimuli have been thoroughly distinguished, mass spectrometric analysis will be done to identify downstream signaling molecules upon binding of individual or both domains.

Conclusions: Ultimately, these studies are expected to render new signaling pathways for CR3. Studying these various pathways will provide fundamental targets for anti-inflammatory and immune enhancing pharmacological therapies.

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G α_{i1} and G α_{i3} Are Required for Epidermal Growth Factor–Mediated Activation of the Akt-mTORC1 Pathway

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The precise mechanism whereby epidermal growth factor (EGF) activates the serine-threonine kinase Akt and the mammalian target of rapamycin (mTOR) complex 1 (mTORC1) remains elusive. Here, we report that the α subunits of the heterotrimeric guanine nucleotide-binding proteins (G proteins) G α_{i1} and G α_{i3} are critical for this activation process. Both G α_{i1} and G α_{i3} formed complexes with growth factor receptor binding 2 (Grb2)-associated binding protein 1 (Gab1) and the EGF receptor (EGFR) and were required for the phosphorylation of Gab1 and its subsequent interaction with the p85 subunit of phosphatidylinositol 3-kinase in response to EGF. Loss of G α_{i1} and G α_{i3} severely impaired the activation of Akt and of p70 S6 kinase and 4E-BP1, downstream targets of mTORC1, in response to EGF, heparin-binding EGF-like growth factor, and transforming growth factor α , but not insulin, insulin-like growth factor, or platelet-derived growth factor. In addition, ablation of G α_{i1} and G α_{i3} largely inhibited EGF-induced cell growth, migration, and survival and the accumulation of cyclin D1. Overall, this study suggests that G α_{i1} and G α_{i3} lie downstream of EGFR, but upstream of Gab1-mediated activation of Akt and mTORC1, thus revealing a role for G α_i proteins in mediating EGFR signaling.

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INSTRUMENTATION AT THE RHODE ISLAND NSF/EPSCoR PROTEOMICS FACILITY

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The Rhode Island NSF/EPSCoR Proteomics Facility, located in the Laboratories for Molecular Medicine at Brown University, houses an assortment of state-of-the-art equipment for the biochemical and biophysical characterization of proteins and other macromolecules. The facility maintains, and offers to the entire Rhode Island research community, the following instruments:

- Jasco J-815 Circular Dichroism Spectrometer with Peltier heating
- MicroCal VP-DSC Differential Scanning Microcalorimeter
- MicroCal VP-ITC Isothermal Titration Microcalorimeter
- Biacore T-100 Surface Plasmon Resonance
- Thermo-Finnigan LTQ Mass Spectrometer with LC-ESI
- Horiba Jobin Yvon FluoroMax-4 Spectrofluorometer
- Qiagen QIAcube
- Infors Minifors Fermentor
- Agilent 1200 series HPLC with Autosampler, UV Detector and Fraction Collector
- GE Healthcare Akta Prime Plus Liquid Chromatography System

Examples of data collected by researchers in the facility will be provided, demonstrating the utility of various instruments. In addition to extending access to the instruments, another mission of the facility is to deliver training to users and consultation on experiments. Training is in the form of online tutorials and hands-on instruction at the facility. Consultations on experimental design and the capabilities of various instruments are offered to all Rhode Island scientists via phone, email and direct meetings.

More information about the facility, including instrumentation, protocols, funding opportunities and a sign-up calendar, are available at http://biomed.brown.edu/epscor_proteomics.

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THE GAIA HIV VACCINE PROGRESS REPORT: BROAD RECOGNITION OF CLASS I AND II-RESTRICTED EPITOPES AND IN VIVO STUDIES

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Problem: Recent HIV vaccine trial failures have made the advantages of an epitope-based vaccine more apparent. Our aim is to develop the “GAIA vaccine”, an epitope-driven, DNA-prime, pseudoprotein-boost HIV vaccine composed of both CTL and T helper cell epitopes that are highly conserved and immunogenic over a broad range of HLA backgrounds.

Methods: 10,199 HIV protein sequences across all clades were searched for conserved 9-10-mer segments. 5,494 (>5%) of the most highly conserved 9-mer sequences were analyzed by EpiMatrix for HLA binding potential and used to create immunogenic consensus sequence (ICS) class II epitopes. Initial evaluations of these epitopes have been performed in Mali and in Providence, RI. DNA-prime/peptide-boost vaccine studies including these epitopes were performed in HLA transgenic mice.

Results: Individual epitopes selected for study are more broadly conserved than those chosen for other previously reported epitope-based vaccines (>70%, compared to Epimmune’s 40%). ELISpots confirmed the immunogenicity of 98% of the ICS epitopes and Class I epitopes (85% A2, 29% A3, 67% B7, 20% A24). Fifteen of the ICS peptides were also confirmed in Mali. Results from two HLA transgenic mouse studies confirmed the immunogenicity of selected epitopes in this model.

Conclusions: Globally conserved, immunogenic HIV epitopes were discovered for class I and II alleles well-represented in the global population. DNA vaccine prototypes encoding these epitopes are immunogenic in HLA transgenic mice. We anticipate that the epitope-rich GAIA vaccine when tested in human volunteers will induce greater immunogenicity than have other DNA/viral vector prime-boost vaccines.

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SMALL SCALE, NOVEL, AGAROSE & MIXED MEDIA BEADS CAN NOW BE DESIGNED FOR SPECIFIC APPLICATION NEEDS

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Problem(s):

- 1) Customized, small-scale agarose beads have not been commercially available; instead, only arbitrary concentrations of 2, 4 and 6% agarose have been provided for SEC chromatography;
- 2) Only a limited range of bead size distributions have been made available low pressure affinity chromatography;
- 3) There has been no means for entrapping functionalized or micronized particles within highly porous beads and
- 4) No customizable magnetic bead alternatives have been available for HIC, IEC, AIC, and other such column separation methods

Methods: U.S. Patent 6, 246, 248 was developed to provide a means to cost-effectively make small volumes of customized agarose beads for individual researchers and specific applications. A wide range of non-integral or “odd” numbered concentrations are available from 1-9 %. These can be made in volumes from 10 ml – liter quantities. Also, both magnetic and functionalized microparticles can be entrapped in agarose beads in order to achieve more rapid bioseparations than possible with columns and pumps.

Conclusions: A wide spectrum of new bioseparation media- including rapid magnetic separations- have been developed which can serve as attractive alternatives to conventional column methods or methods based only on “off the shelf” or generic products. Bioscience research scientists can therefore now collaboratively design bioseparation beads which exactly suit their application- even if such media has never before been created.

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NATURAL REGULATORY T-CELL EPITOPE INDUCTION IN AUTOIMMUNE EAE SUGGESTS NEW THERAPY FOR MULTIPLE SCLEROSIS

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Foundation

Problem: Immunoglobulin (IgG) therapy expands regulatory T cells and protects against disease development in experimental autoimmune encephalitis (EAE, Ephrem et al., Blood 111:715-22, 2008). We have identified sequences within IgG that activate natural regulatory T cells, Tregitopes. We hypothesize that protection is mediated by the activation of regulatory T cells specific for these IgG epitopes (Tregitopes).

Methods: The studies were performed two ways using the MOG peptide mouse model: 1) Tregitopes were delivered concurrently with MOG at disease induction (subcutaneously) and then ip for 5 additional days. 2). Tregitopes were administered for 5 days i.p at the time of disease onset. Finally, we investigated liposomes as a possible delivery method to enhance the effects of Tregitope treatment.

Results: Symptoms were initially suppressed with Ig derived regulatory T-cell epitopes, which suggests possible therapeutic effects; however by day sixty, disease progression was indistinguishable in all groups. Analysis showed that Tregitope treatments delayed onset of disease, reduced severity of symptoms when administered at the peak of disease and mice vaccinated with Tregitopes delivered by liposomes had an overall decrease in disease severity.

Conclusions: The discovery of natural regulatory T-cell epitopes in the Fc fragment of IgG may lead to disease-management paradigm shift. This may permit T cells to regulate immune responses in a wide range of health conditions such as multiple sclerosis.

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TARGETING LYMPHOMA WITH BISPECIFIC ANTIBODY-ARMED ACTIVATED T CELLS AND POTENTIAL APPLICATION IN ALLOGENEIC TRANSPLANT

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New approaches are desperately needed for patients with high risk-refractory or relapsed lymphomas, for whom prognosis is poor and therapeutic options limited. Current treatment strategies combining chemotherapy with rituximab (anti-CD20 monoclonal antibody) or high-dose chemotherapy (HDC) with allogeneic stem cell transplant (alloSCT) yield serious toxicities and unacceptably high relapse rates. In particular, the anti-lymphoma benefits of alloSCT are grossly limited by life-threatening graft vs host disease (GvHD). Thus, novel approaches to increase the graft vs lymphoma effect (GvL) without increasing GvHD are necessary to improve the disease-free survival and quality of life for these patients. Bispecific antibodies (BiAbs) are produced by linking anti-CD3 for T cell binding to an antibody specific for a tumor antigen. When T cells are activated and “armed” with BiAb, every T cell possesses tumor-specific cytotoxic function. Because this activity is independent of further activation, proliferation, antigen presentation or costimulation *in vivo*, we hypothesize that armed activated T cells (ATC) will provide greater GvL than conventional unmanipulated T cells, without proportional increases in GvHD. Studies here examined allo-reactivity of BiAb-armed ATC *in vitro* for allogeneic applications. Data show that ATC armed with anti-CD3 x anti-CD20 BiAb specifically target and kill B cell lymphomas. However, BiAb-armed ATC are remarkably poor stimulators of, or responders to, allogeneic stimulation. These results provide important evidence of a functional separation between the GvL and GvH effects of BiAb-armed ATC. Translation of these results into clinical applications will provide an important and much needed advances over current strategies available to these patients.

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***UTH1* Regulates the Integrity of the Yeast Cell Wall by Altering Its Polysaccharide Content**

Deletion of the gene, *UTH1*, a founding member of the SUN family of fungal genes, has pleiotropic effects. Several phenotypes of *Duth1* cells including their decreased levels of mitochondrial proteins, their impaired autophagic degradation of mitochondria, and their increased viability in the presence of mammalian BAX, a pro-apoptotic regulator localized to the mitochondria, have prompted others to propose that the *UTH1* protein functions primarily at the mitochondria. In this report, we show that *UTH1* regulates the integrity of the yeast cell wall by altering its polysaccharide content. Specifically, cells lacking *UTH1* have more robust cell walls that are resistant to zymolyase treatment. Their cell walls also contain higher levels of β -D-glucan and lower levels of chitin. Furthermore, our data suggests that several of the enhanced growth phenotypes of *Duth1* cells, including their resistance to BAX-mediated toxicity, arise from a strengthened cell wall. Thus, we propose that *Uth1p*'s role at the cell wall and not at the mitochondria may better explain many of its effects on yeast physiology and programmed cell death.

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An Approach to Change in the Field of Chemical Dependency and Addiction

Desjarlais, J.L., Russo, E., Warot, S.S., Montvilo, R.K. (2009)

Department of Psychology, Rhode Island College, Providence, RI
RI-INBRE Summer Undergraduate Research Fellowship Program

Many institutions assist addicted individuals with their recovery, but many individuals fall through the cracks. Problems are encountered with accessing the system, with service providers, within the judicial system, in the health care system or in the legislative system. By learning about services available (and lacking), we hope to foster positive change within the Chemical Dependency field. In the summer of 2009, we learned about the framework of the continuum of care within this field. While there are many resources available, there is a lack of continuity among components of the system. There needs to be consideration given to special populations such as women, developmentally disabled, and the elderly. Identification of obstacles specific to each population as well as those common across the spectrum, may help bridge gaps serving as barriers to recovery. Behavioral health professionals often avoid implementing evidence-based research into practice. Many practitioners are exposed to evidence-based research only through continuing education, and attend only when required to, using these sessions to socialize rather than to learn. Thus, addiction research remains largely isolated from those it is intended to inform. Two experiments using qualitative and quantitative data are being performed to determine whether continuing education courses for addiction counselors will have greater impact if they are presented on-line rather than in the typical conference format. By implementing an on-line modality as a continuing education option, we hope to bridge the gap between the findings of effective treatment in evidence-based practice, to the actual dissemination of knowledge to the providers.

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CARDIAC MYOCYTES AND FIBROBLASTS IN A NOVEL THREE-DIMENSIONAL CULTURE SYSTEM

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Problem: Cardiac myocytes (CM) and fibroblasts (CF) determine cardiac structure and function. While conventionally studied in two-dimensional monolayer cell cultures, CM and CF in three-dimensional (3D) cultures are exposed to cell-cell and cell-matrix interactions, electrical coupling, and a cardiac tissue-like morphology. This study was designed to create a novel 3D culture system which can produce large numbers of self-assembled microtissues and allow for fine control of their size, medium exchange, and visualization.

Methods: Neonatal rat CM and CF were seeded alone or together in non-adhesive hydrogels containing 822-recess (800- μ m deep, 400- μ m wide). Cells settled into the recesses (~1800 cells/recess) under gravitational force and self-assembled into spheroid-shaped microtissues. Parameters including cell viability/cytotoxicity, morphology, self-assembly, contraction, and distribution were used to evaluate cell behavior.

Results: The viability/cytotoxicity staining showed that CM and CF formed viable spheroids with non-necrotic centers and were most viable in serum-containing media. Self-assembly was comparable when CM and CF were plated alone, but was slightly faster in heterotypic spheroids. Spontaneous contractions were observed 24 hours after plating in spheroids containing CM. CM and CF show an *in vivo*-like interspersions in heterotypic spheroids, monitored by fluorescent-labeling. Cells within spheroids can be infected successfully with adenoviruses without impacting aggregation or viability, indicating the capability of spheroids for gene transfer.

Conclusions: This novel 3D culture system generates a large number of cardiac microtissues, which can be easily created, visualized, and studied. These spheroids more closely resemble cardiac tissue *in vitro*, and can be used to study cell signaling, function and communication.

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IMMUNOINFORMATIC-DISCOVERED *MYCOBACTERIUM TUBERCULOSIS* T-CELL EPITOPES ARE IMMUNOGENIC IN HLA TRANSGENIC MICE

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Problem: Generation of a multi-epitope *Mycobacterium tuberculosis* (Mtb) vaccine that targets latently-infected individuals. Here, we describe proof-of-concept studies to identify TB vaccine candidates.

Methods: EpiMatrix, an algorithm for T-cell epitope analysis was used to screen published antigenic and protective Mtb antigens. Several Class II HLA-binding motifs bearing low human sequence homology were selected for an in vivo immunogenicity study. Through liposomal delivery, HLA A2/DR1 transgenic mice were immunized intranasally (2 doses) with epitope peptides and adjuvant. Immunogenicity was measured by ELISpot and ELISA.

Results: EpiMatrix DR1 predictions were statistically significant ($p < 0.001$). 58% of A2/DR1 transgenic mice vaccinated with peptide displayed Class II HLA and individual epitope T cell responses as well as increased IL-2 production. The concentration of HLA binding motifs to the peptides in HLA transgenic mice influenced immunity.

Conclusions: Computational epitope mapping identifies Mtb sequences that are immunogenic and capable of eliciting a multi-cytokine response in a humanized mouse model. Analysis of in vivo and predicted immunogenicity for individual epitopes reveals a correlation for both DR1 and non-DR1 epitopes. A heterologous DNA-prime/peptide-boost strategy will be adopted to elicit the maximum immune response.

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THE POST-GLACIAL HISTORY OF THE BARNACLE, *SEMIBALANUS BALANOIDES*, IN THE NORTHWEST ATLANTIC: NEUTRAL AND SELECTIVE POPULATION GENETIC PROCESSES

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Problem: The population genetic structure of organisms is shaped by the life history characteristics of the species and the geologic history of their habitat. Understanding the ecological roles of a species requires a thorough assessment of how its distribution and abundance have evolved through time. The ecological foundation species, *Semibalanus balanoides*, is used to study community interactions, environmental change and the genetics of selection, but no comprehensive study of neutral population substructure exists in this species.

Methods: Neutral markers, including microsatellites and mtDNA, were surveyed in barnacles collected along an 800 km transect of the western Atlantic coast. Population genetic structure was assessed in the context of the complex glacial history of the region. A putatively selected locus, *Mannose phosphate isomerase (Mpi)*, was also sequenced in populations across this range to look for evidence of non-neutrality.

Results: Barnacle populations show population genetic patterns characteristic of glacial vicariance and recolonization, including decreased diversity with increased latitude and an excess of high frequency polymorphisms in DNA sequence data. Significant isolation by distance was observed along the coast. Sequence evolution at the *Mpi* locus was also consistent with glacial vicariance.

Conclusions: This study provides a critical neutral framework for future assessments of the ecological roles of *Semibalanus balanoides*. The added genetic variance produced by glacial events may represent an important source of selectable variation at the *Mpi* locus, and across the genome of this species.

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Abundance, Growth, and Diet of Juvenile Summer Flounder (*Paralichthys dentatus*) and Winter Flounder (*Pseudopleuronectes americanus*) In the Seekonk River, RI and the Taunton River, MA.

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Problem: Summer flounder, *Paralichthys dentatus*, and winter flounder, *Pseudopleuronectes americanus* utilize estuaries as nursery habitat during their early life history stages. In southern New England estuaries, however, little is known regarding the spatiotemporal overlap and potential biotic interactions between the flounder species. The purpose of this research was to assess the abundance, growth, and dietary habits of juvenile summer and winter flounder to determine if predator-prey and/or competitive relationships exist.

Methods: From May to August 2009, flounder in the Seekonk River (5 sites) and Taunton River (6 sites) were sampled biweekly using beach seines. Captured flounder were enumerated, measured for total length (mm), and a sub-sample was preserved for subsequent stomach content analysis.

Results: Summer flounder abundance (mean = 0.54 fish/m²) decreased significantly over time, but the abundance of winter flounder (mean = 0.30 fish/m²) remained relatively constant during the sampling period. Summer flounder grew significantly faster than winter flounder (growth rates = 0.98 and 0.49 mm/day, respectively), which may be attributed to differences in dietary habits. Decapods and fish were an important component of the summer flounder diet (44% and 5% by volume, respectively), while amphipods and nematodes were favored by winter flounder (35% and 25% by volume, respectively).

Conclusions: These data suggest that competition for food resources is minimal between species. Among the identifiable fish prey in summer flounder stomachs, however, there was evidence of predation on winter flounder, albeit to a limited extent.

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Case Study: Use of Growth Colony Stimulating Factor (GCSF) for the Healing of Chronic Wounds in Humans

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Problem: Chronic wounds affect millions of Americans and show little if any response to a variety of treatment modalities. Thus, an attractive idea is to treat these wounds with a younger population of cells that would be more responsive to growth factors. Therefore, our objective was to determine whether bringing a patient's own (autologous) stem cells to their chronic wound would lead to healing.

Methods: Stem cell mobilization was accomplished via SQ injections of GCSF, for 4 consecutive days, with a dose of 10ug/kg. Followed by fibrin spray to the wound itself. In addition, the subject received local wound care and compression therapy. Blood was drawn, at various time points, to check for the presence of stem cell markers and blood count. In addition, biopsies of the wound and thigh were performed for histological examination. The subject was followed on a weekly basis for 24 weeks. At each visit, the wound was examined, traced for planimetry, and photographed.

Results: One 63 y/o Caucasian male with a chronic venous leg ulcer was enrolled and completed the treatment phase. The subject's white blood cell count rose from 8.2 (screening) to 42.6 (after 3 injections). Stem cell markers CD34 and CD117 increased between the 1st and 3rd GCSF injection. The wound size decreased from 7.9 cm² (screening) to 3.7cm² 24 weeks s/p injections. Clinically, the wound became more superficial and granulation tissue increased.

Conclusions: Early data shows GCSF improves the clinical appearance of chronic wounds. More subjects and data are needed.

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WHOLE-GENOME CHROMOSOME COPY NUMBER CHANGES IN FANCONI ANEMIA

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Problem: Fanconi anemia (FA) is a recessive disease characterized by congenital abnormalities, bone marrow failure and cancer susceptibility. The FA proteins, and the major breast cancer susceptibility gene products FANCD1/BRCA2 and BRCA1, function cooperatively in the FA-BRCA pathway to repair damaged DNA. Several studies have suggested an important role for the FA-BRCA pathway in the maintenance of chromosome stability during DNA replication. Nevertheless, the molecular basis for the increased cancer susceptibility observed among FA patients is poorly understood. We hypothesize that the FA-BRCA pathway plays a major role in the prevention of sub-microscopic chromosome copy number changes (CNCs) throughout the genome that arise as a consequence of DNA replication stress. These mutational events may be highly relevant to cancer initiation and progression in FA patients.

Methods: To test this hypothesis we used array comparative genomic hybridization (aCGH), to determine the frequency and rate of occurrence of *de novo* CNCs in FA cells, incubated in the absence or presence of the DNA polymerase inhibitor aphidicolin (APH).

Results: Compared with normal cells, FA cells acquire both spontaneous and APH-induced *de novo* CNCs at a higher rate and frequency. The CNCs detected overlap with known cancer-associated pathogenic CNCs, and include deletions and duplications ranging in size from ~12 kb up to 28 Mb.

Conclusions: Our preliminary results support the hypothesis that the FA-BRCA pathway plays a major role in the prevention of genome-wide pathogenic CNCs. Determining the origins of these CNCs may have direct relevance to improving therapeutic options for FA.

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Promise of Embedded System with GPU in Artificial Leg Control: Enabling Time-frequency Feature Extraction from Electromyography

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Problem: Electromyography (EMG) pattern recognition has been successfully applied to control multifunctional artificial arms, while applying EMG pattern recognition to artificial leg control is challenging because leg EMGs are non-stationary. Time-frequency (TF) features are suitable for representing non-stationary signals in pattern recognition; however, the computation complexity to extract TF features is too high such that current embedded systems applied for artificial limb control are inadequate for real-time computing. The aim of this study was to quantify the computation speed of a novel high performance embedded system, Graphic Processor Unit (GPU), on EMG TF feature extraction.

Methods: EMGs of thigh muscles were collected from an able-bodied subject while walking. EMG TF feature extraction included the Hilbert transformation and time-frequency transformation of each EMG signal in each analysis window. Principle component analysis was used to reduce the dimension of TF feature vectors. The computation times for these analytic algorithms were measured on both a commodity embedded system with a GPU and a general purpose PC.

Results: The results showed up to two orders of magnitude speedup of the computation on the GPU system as compared to the CPU architecture.

Conclusions: High performance GPU shows a great promise for real time and high speed computation for EMG TF feature extraction and eventual application to EMG-controlled artificial legs. With continued advancement in performance and decrease in cost, GPU may be applied to other biomedical applications that need high-speed and real-time computation.

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STUDY OF STUMBLE RECOVERY FOR ABLE-BODIED AND TRANSFEMORAL AMPUTEE SUBJECTS

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Problem: Current lower limb prostheses are not fully robust, resulting in falls that could cause serious injuries in leg amputees. The goal of this project is to study and compare the stumble recovery patterns between able-bodied subjects and subjects with transfemoral amputations. The results can lead to the development of responsive stumble detection and new prosthesis control for active stumble recovery, which will improve the safe use of artificial legs.

Methods: Five unilateral transfemoral amputees and ten able-bodied subjects were recruited for this study. Subjects walked on a treadmill, which simulates slipping and tripping in a controlled setting. Ground reaction force (GRF) and kinematics of both lower limbs (including the prosthetic leg for amputee subjects) were collected. Surface EMGs were recorded from one side of the lower limb or the residual limb.

Results: Differences between the normal gait patterns and those after the tripping/slipping events were studied. Visible pattern changes in kinematics and GRF after the perturbations were within 30 ms, which appeared earlier than those in EMG envelop. Subjects with transfemoral amputation showed unique recovery methods depending on their mobility and the type of knee units in their prostheses.

Conclusions: The outcome of this study show potential for accurate and responsive stumble detection. Our future work includes design of stumble detectors and prosthesis control for active stumble recovery, which will establish improved safety during prosthesis use and enhances the quality of life for patients with lower-limb amputations.

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ECM PROTEIN MATN3 SHOWS POTENTIAL TO SPECIFICALLY BIND AND POTENTIALLY INHIBIT ACTIVITY OF CERTAIN CATABOLIC INFLAMMATORY CYTO/CHEMOKINES

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Problem: OA is characterized by its degenerative effect on articular cartilage extracellular matrix (ECM) structure. It is one of the most common types of arthritis and affects an estimated 33 million Americans who are past middle-age. It is currently known that aging and extensive mechanical loading of articular cartilage are leading factors that can result in OA development; however, the exact cause of articular cartilage degradation is not well understood. MATN3, a member of the matrilin family of extracellular matrix proteins, has been demonstrated to potentially prevent the early onset of OA; therefore it is chondroprotective. We hypothesize that the chondroprotective nature of MATN3 may be attributed to its possible ability to bind inflammatory cytokines and/or chemokines resulting in sequestering of these factors in the matrix and reducing their accessibility to the cells.

Methods: Solid Phase Binding Assay (SPA) was performed to screen for binding interactions between MATN3 and the cyto/chemokines: IL-1 α , IL-6, TNF- α , and SDF-1 β . Surface Plasmon resonance (*Biacore*) kinetics binding assay was conducted between MATN3 and SDF-1 β .

Results: Dose-dependent binding between MATN3 with IL-6 and SDF-1 β was observed. IL-6 to MATN3 binding is concentration dependant. Biacore kinetics run also confirms that MATN3 binds SDF-1 β in a dose-dependent manner.

Conclusions: This research demonstrates that MATN3 can specifically bind certain inflammatory cyto/chemokines (IL-6 and SDF-1 β). This phenomenon suggests that MATN3 has the natural capability to sequester certain catabolic inflammatory cytokines perhaps preventing them from reaching their receptor targets. This may be the mechanism by which MATN3 acts chondroprotectively.

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CARDIAC MYOCYTE BEHAVIOR IN MICROPATTERNED AND CONTINUOUSLY PACED SHORT-TERM CULTURES

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Problem: In the heart, cardiac myocytes (CM) are oriented along an axis and exposed to rhythmical electrical stimulation. Two-dimensional (2D) monolayer cell cultures are often used to study CM behavior, but spreading in random orientation with no electrical stimulation may change cell behavior. This study was designed to incorporate both micropatterning and continuous electrical field stimulation (CES) to advance conventional 2D cultures and to test whether the resulting CM would resemble more closely those in the myocardium.

Methods: Neonatal rat CM were plated onto glass coverslips with microcontact printing of fibronectin in long stripes (10 mm) of variable widths (50-450 μ m). Additionally, some cells were paced at 5 V/cm. Phenylephrine and timolol were used to investigate hypertrophic responses. CM alignment, morphology, sarcomeric organization, cell-cell connections, and hypertrophy were assessed by phase contrast and fluorescent microscopy using antibodies against α -sarcomeric actinin, connexin 43 (Cx43), and atrial natriuretic factor (ANF).

Results: CM selectively attached to micropatterned stripes. Unlike conventional culture, they were more elongated with increased sarcomeric organization. The phenotype of CM was more pronounced at the protein/glass border than in the middle of stripes. Patterned CM with CES showed a further increase in alignment, elongation, sarcomeric organization and Cx43 expression versus unpaced cells, particularly when current was applied parallel to the pattern. Phenylephrine caused an increase in cell size, ANF expression, sarcomeric organization and Cx43 expression in patterned CM with or without CES.

Conclusions: The phenotype of CM in micropatterned and electrically stimulated 2D cultures more closely resembles those in tissue.

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Supv3L1 Mouse: A Model for Aging and Skin Defects

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Supv3L1 is a conserved and ubiquitously expressed helicase found in numerous tissues and cell types of many species. In human cells, SUPV3L1 was shown to suppress apoptotic death and sister chromatid exchange, and impair mitochondrial RNA metabolism and protein synthesis. In vitro experiments revealed binding of SUPV3L1 to BLM and WRN proteins, suggesting a role in genome maintenance processes. Disruption of the Supv3L1 gene in the mouse has been reported to be embryonic lethal at early developmental stages. We generated a conditional mouse in which the phenotypes associated with the removal of exon 14 can be tested in a variety of tissues. Disruption mediated by a Mx1 promoter-driven Cre displayed a postnatal growth delay, reduced lifespan, loss of adipose tissue and muscle mass, and severe skin abnormalities manifesting as ichthyosis, thickening of the epidermis, and atrophy of the dermis and subcutaneous tissue. Using a tamoxifen-activatable Esr1/Cre driver, Supv3L1 disruption resulted in growth retardation and aging phenotypes, including loss of adipose tissue and muscle mass, kyphosis, cachexia, and premature death. Many of the abnormalities seen in the Mx1-Cre mice, such as hyperkeratosis characterized by profound scaling of feet and tail, could also be detected in tamoxifen-inducible Cre mice. Conditional ablation of Supv3L1 in keratinocytes confirmed atrophic changes in the skin and ichthyosis-like changes. Together, these data indicate that Supv3L1 is important for the maintenance of the skin barrier. In addition, loss of Supv3L1 function leads to accelerated aging-like phenotypes.

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IL10 ameliorates hypoxia induced pre-eclampsia-like symptoms via negative regulation of P53 signaling

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Problem: the etiology of preeclampsia remains largely unknown despite extensive clinical investigation of severe health risks to the mother and the fetus. Based on morphologic changes and *in vitro* studies on placental tissue, hypoxia is thought to be one of the key pathologic manifestations associated with preeclampsia. However, *in vivo* evidence for hypoxia-induced pathology is lacking.

Methods: real-time quantitative PCR, western blot, immunohistochemistry, immunofluorescence and ELISA.

Results: hypoxic perturbation in wild type (WT) and IL-10^{-/-} mice beyond early stages of pregnancy induced preeclampsia-like disease, including high systolic blood pressure, proteinuria, fetal intrauterine growth restriction (IUGR) and kidney pathology. These hypoxia-induced features were not observed in age-matched non-pregnant mice. Interestingly, hypoxic environment caused elevated presence of anti-angiogenic factors such as soluble Flt1 (sFlt1) and sEndoglin (sEng) in pregnant mice. Hypoxia-induced pathology was greatly exacerbated in IL-10^{-/-} mice, suggesting a protective role of IL-10 against hypoxia during pregnancy. The severity of preeclampsia-like features in IL-10^{-/-} mice was found to be associated with dramatically elevated expression of p53 and pro-apoptotic protein Bax, down-regulation of Bcl-2, and trophoblast-specific apoptosis in utero-placental tissue. Graded alteration in the expression of Bax and Bcl-2 in WT and IL-10^{-/-} mice showed a HIF-1 α -independent profile.

Conclusion: Taken together, we present *in vivo* evidence for hypoxia-induced preeclampsia-like disease and conclude that IL-10 provides partial protection against the disease. Placental IL-10 deficiency may prove to be a trigger for preeclampsia and consequently, IL-10 may also serve as a therapeutic modality to prevent or treat this enigmatic pregnancy condition.

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Second Hand Smoke Exposure During Pregnancy Influences Associations between Placental Genes Regulating Stress Response: A Pilot Study

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Problem: Exposure to second hand smoke (SHS) contributes to health complications in both adults and children, few studies have examined the effect that SHS exposure has on a developing fetus. Preliminary studies have shown that an altered intrauterine environment influences placental norepinephrine transporter (NET), 11beta-hydroxysteroid dehydrogenase type 2 (11 β HSD2) and serotonin transporter (SERT) gene expression. We hypothesized that exposure to SHS will lead to a down regulation of NET, 11 β HSD2 and SERT in the placenta, mimicking effects seen with prenatal smoking.

Method: Placenta samples were collected from 31 participants in an ongoing prospective study. Six mothers had been exposed to SHS (Av: 27.5 hrs), 14 mothers smoked and 11 mothers were non-smokers and denied exposure to SHS. Smoking status and level of SHS exposure were determined by *Time Line Follow Back* and a hospital chart review. Risk scores were calculated for each using a pre-established 13 point formula. Placental samples were placed in *RNAlater™*, frozen at -80°C, RNA was extracted and cDNA made for RT-qPCR with hNET, h11 β HSD2 and hSERT gene-specific primers/probes.

Results: We observed a statistically significant decrease in 11 β HSD2 expression with increasing risk score ($p < 0.0456$) and also a significant difference in the controls compared to the SHS mothers (0.0092). SERT was also significantly reduced when correlated with risk score ($p < 0.0026$).

Conclusion: Second-hand smoke exposure during pregnancy is associated with down regulation of placental NET, 11 β HSD2 and SERT gene expression. As hypothesized, effects of SHS exposure are similar to those from direct maternal smoking.

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STEM CELL MOBILIZATION WITH SYSTEMIC GCSF APPEARS TO IMPROVE MOUSE AND HUMAN WOUND HEALING

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Problem: We sought to stimulate the healing of difficult-to-heal wounds using stem cells. Granulocyte colony stimulating factor (GCSF) is the best cytokine to mobilize stem cells from the bone marrow to the peripheral blood. We studied the effect of GCSF on wounds in mice and in a human subject.

Methods: After administering GCSF to mice, we performed flow cytometry and a colony forming assay to determine the number of progenitor cells in the peripheral blood at different time points. We monitored the healing of mouse tail wounds following GCSF injections, and we measured the rate of re-epithelialization and connective tissue formation. Chimeric mice with green fluorescent protein (GFP) bone marrow cells were treated with GCSF and wounded. We then followed the homing of these GFP cells to the wound. One human subject has thus far been enrolled in a study on the effects of GCSF on human chronic wounds refractory to standard care. This patient has been followed by clinical and histological examination.

Results: Experimental mouse tail wounds showed better quality of healing with GCSF, though neither the re-epithelialization assay nor the connective tissue assay was sensitive enough to show a statistically significant difference. Bone marrow-derived cells accumulated at the wound after one day of wounding, but the pending histological results are needed to identify these cells. The wound of the human subject treated with GCSF also showed promise, with better granulation and epithelialization.

Conclusion: GCSF stem cell therapy shows promise in mouse and human wound healing.

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PHAGOCYTOSIS OF *CANDIDA PARAPSILOSIS* BY HUMAN NEUTROPHILS IS MEDIATED THROUGH MANNAN, POSSIBLY VIA GALECTIN-3.

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PROBLEM: *Candida parapsilosis* has begun to emerge as a significant source of hospital acquired infections, however little is known about the host-pathogen interaction. This study focused on the interaction of *C. parapsilosis* with human neutrophils, a key mediator against fungal infections. The goal of this study was to determine the fungal ligand and the neutrophil receptor responsible for phagocytosis of *C. parapsilosis* by neutrophils.

METHODS: Phagocytosis of *C. parapsilosis* by neutrophils was evaluated by fluorescence microscopy using a previously established assay. To determine if mannan, a fungal cell wall component, played a role in phagocytosis, neutrophils were treated with an excess amount of *C. parapsilosis* mannan. To determine if the lectin receptor galectin-3 played a role in phagocytosis, neutrophils were treated with a blocking antibody against galectin-3.

RESULTS: Under control conditions, neutrophils phagocytosed *C. parapsilosis* with high efficiency. When neutrophils were treated with excess mannan, phagocytosis of *C. parapsilosis* was attenuated. Phagocytosis of *C. parapsilosis* was also reduced when neutrophils were treated with the galectin-3 blocking antibody.

CONCLUSIONS: Attenuation of phagocytosis with mannan treatment indicates that mannan may be a fungal ligand involved in phagocytosis. Reduction of phagocytosis with a galectin-3 blocking antibody is consistent with *C. parapsilosis* mannan being the fungal ligand responsible for inducing neutrophil phagocytosis as galectin-3 binds β -(1-2)-mannose, a component of fungal mannan. Further studies are underway to 1) determine the structure of *C. parapsilosis* mannan and 2) identify direct interactions of *C. parapsilosis* yeast and mannan with galectin-3.

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Estuarine invertebrates and forage finfish as bio-indicators of environmental mercury levels in the Narragansett Bay (Rhode Island, USA)

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Estuarine environments are susceptible to anthropogenic perturbations, including the deposition and mobilization of a variety of contaminants in the sediment. Environmental mercury (Hg) contamination is of particular concern because Hg bioaccumulates in aquatic food webs and exposure has deleterious effects on biota, including humans. The potential health risks associated with Hg exposure justifies the development of monitoring programs that link environmental and biological Hg contamination. In a preliminary study, we assessed the utility of estuarine invertebrates and forage finfish as bio-indicators of environmental Hg pollution. Specifically, surface sediments (0-2 cm; 54 sites) and biota (finfish, macrocrustaceans, bivalves, polychaetes, and zooplankton; 83 sites) were collected from the Narragansett Bay (Rhode Island), and analyzed for total Hg concentration using atomic absorption spectroscopy. Spatial relationships between sediment and biota Hg concentrations were then statistically compared using Geographic Information System and least-squares linear regression models. There was a significant positive correlation between sediment Hg levels and the Hg content of bivalves ($R^2 = 0.389$; $p < 0.0006$) and zooplankton ($R^2 = 0.2613$; $p < 0.005$). Conversely, polychaete, macrocrustacean, and finfish Hg body burdens were not significantly related to environmental Hg levels ($R^2 = 0.091, 0.322, 0.012$; $p = 0.2746, 0.1463, 0.5236$). Preliminary results therefore indicate that the effectiveness of estuarine biota as bio-indicators of environmental Hg contamination is taxon-specific, and is likely influenced by feeding ecology, longevity, and site fidelity.

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DEVELOPMENT OF ELECTROLYTES FOR LITHIUM ION BATTERIES

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Problem: Due to their high energy density, high discharge voltage, and long cycle life, lithium-ion batteries (LIBs) are widely used for small electronic devices. However, currently available LIB technology does not satisfy some of the performance goals for Electric Vehicles (EV) due to loss of power and capacity upon storage or prolonged use, especially at moderately elevated temperatures ($>55\text{ }^{\circ}\text{C}$). The performance degradation is linked to the thermal stability the electrolyte.

Methods: We have developed a novel lithium electrolyte, lithium tetrafluorooxalatophosphate ($\text{LiPF}_4\text{C}_2\text{O}_4$). The electrochemical performance of carbonate solutions of $\text{LiPF}_4(\text{C}_2\text{O}_4)$ were investigated via cyclic voltammetry and electrochemical cycling. After initial cycling, we investigated the surface of the electrodes with a combination of IR-ATR, XPS, and SEM to determine role of the electrolyte in Solid Electrolyte Interface (SEI) formation and stability.

Results: Carbonate solutions of $\text{LiPF}_4\text{C}_2\text{O}_4$ have similar conductivity to LiPF_6 , but are thermally stable. $\text{LiPF}_4(\text{C}_2\text{O}_4)$ electrolytes generate stable SEI. Further development of the synthesis of $\text{LiPF}_4\text{C}_2\text{O}_4$ may yield a novel low cost thermally stable electrolyte for lithium ion batteries.

Conclusions: The thermal stability of $\text{LiPF}_4(\text{C}_2\text{O}_4)$ /carbonate electrolytes is much better than LiPF_6 /carbonate electrolytes (the state-of-art electrolyte). Incorporation of $\text{LiPF}_4(\text{C}_2\text{O}_4)$ electrolytes into lithium-ion cells provides better capacity retention after accelerated aging experiments. Ex-situ surface analysis of the electrodes after accelerated aging suggests differences in structure of the SEI. $\text{LiPF}_4(\text{C}_2\text{O}_4)$ /carbonate electrolytes are a promising alternative for lithium-ion batteries in EV applications.

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BISPECIFIC ANTIBODY APPROACHES TO ENHANCE PULMONARY STEM CELL ENGRAFTMENT

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Problem: The success of stem cell based regenerative therapies to treat pulmonary diseases has been limited by low stem cell engraftment efficiency. Bispecific antibody (BiAb) technology, based on two heteroconjugated monoclonal antibodies, has been used to increase effector cell delivery to target tissues. *We hypothesized that stem cells armed with a bispecific antibody specific for respiratory epithelium may target intranasally delivered stem cells more efficiently to the respiratory epithelium, thereby enhancing engraftment.* The objective of this study was to develop and employ a novel bispecific antibody to deliver CD34+ human umbilical cord blood-derived hematopoietic progenitor cells to murine VCAM-expressing respiratory epithelium.

Methods: VCAM-1 expression in a murine lung epithelial (MLE-12) cell line was assayed by flow cytometry. Anti-human CD34 and anti-mouse VCAM-1 antibodies were heteroconjugated by standard methods. Heteroconjugation was validated by SDS-PAGE. Arming efficiency was determined using MLE-12 cells armed with BiAb. *In vivo* effects were assessed by intranasal delivery to newborn mice (2×10^5 cells/pup; analysis 2 weeks post-transplantation).

Results: Robust VCAM expression was confirmed in MLE-12 cells. The BiAb resolved as 25% dimer (active), 6% multimer (active) and 69% monomer (inactive) fractions. Flow cytometry revealed 94.8% arming efficiency at 500 ng BiAb/ 10^6 MLE-12 cells. *In vivo* delivery of armed CD34+ cells to newborn mice was well tolerated (lung analyses pending).

Conclusion: We developed and validated a novel hCD34xmVCAM-1 BiAb. Analysis of *in vivo* studies will determine whether pulmonary engraftment efficiency is amplified and therefore whether BiAbs can be considered a valid adjunct treatment for injured lung tissues.

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Allogeneic Bone Marrow's Paracrine Capacity Initiates Angiogenesis in Human Islets Co-Culture Systems

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Introduction: We had previously shown that allogeneic bone marrow (BM) supports human islets survival and function in long-term (over three years) cultures. Since human islets reconstitute a micro-organ sensitive to vascular supply, we hypothesized that BM initiating angiogenesis is one of the mechanisms by which BM supports human islets survival and function.

Methods: Human islet and human BM were co-cultured in RPMI-1640 with 10% fetal bovine serum. Islet function was evaluated by quantifying insulin with ELISA kit. Islet vascularization was identified by fluorescent immunohistochemistry and photographed using a confocal fluorescence microscope. Human Angiogenesis microarray was performed by Taslon Biotechnologies Company using human angiogenesis microarray kit.

Results: We found that BM stimulates human islet angiogenesis. Using immunohistochemistry, we observed significant endothelial distribution in the entire islets co-cultured with BM but no angiogenesis in islets cultured without BM. We also found significantly higher levels of VEGF- α and PDGF but lower levels of angiopoietin-2 in the media of human islets co-cultured with BM vs. islet only culture by using protein array suggesting that BM paracrine function may play a critical role. Cocultured human islets were also found to have significantly higher gene expressions for insulin, glucagon and somatostatin than islet only culture.

Conclusion: Bone marrow has paracrine capability which can initiate human islet angiogenesis in vitro to support survival and function of human islets.

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Effects of Ginsenosides Rb2 on pancreatic β cell oxidation stress.

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Introduction: Ginseng root displays the ability to achieve glucose homeostasis both experimentally and clinically, but lack of knowledge for ginseng on pancreatic β cells hampers its application. Previously, we demonstrated that certain ginsenosides Rb2 and Re but not Rg1 and Rd can aid islet function. We hypothesize that ginsenosides, Rb2, may promote β cell function through anti-oxidation dependent mechanism.

Methods: Rat pancreatic β cell derived cell line, INS-1 cell, was cultured in RPMI-1640 with 10% fetal bovine serum and 50 mM 2-mercaptoethanol to maintain differentiation. Effects of Rb2 on β cell function were tested in Streptozotocin (STZ) treated INS-1 cell culture. Cell viability and function were evaluated by Apoptotic assay (TUNEL) and insulin ELISA kit. Oxidation was evaluated by OxiSelect™ Oxidative DNA Damage ELISA Kit.

Results: Various concentration of STZ (4, 8 and 12 μ M) treated INS-1 cells for 48 hours reduced cell insulin releasing (53%, 49% and 37% respectively vs. control, $p < 0.05$). However, Rb2 (5, 10 ng/ml) revised STZ (10 μ M) induced insulin reduction (71.8 and 75.6ng/ml vs. STZ 37 *ng/ml, $p < 0.05$). STZ induced INS-1 cell oxidation has been clarified (Oxidation level 6.7 ng/ml vs. control 6.2 ng/ml). Rb2 (5 and 10 ng/ml) significantly reduces STZ (10 μ M) induced cell oxidation levels (6.2* and 6.0* ng/ml vs. STZ 6.7 ng/ml $p < 0.05$). In addition, Rb2 (10 ng/ml) increased β cell viability by preventing cell from apoptosis. Apoptosis rate of control, Rb2, STZ and RB2 + STZ are 30.0% \pm 1.09%, 33.45% \pm 0.6%, 97.5% \pm 0.6% and 31.4% \pm 6.7% respectively ($p < 0.0001$ vs. STZ group).

Conclusions: This study demonstrates that Rb2 can protect β cells from STZ induced dysfunction through prevent cell oxidation stress and apoptosis.

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Quantification of Cultured Adult Human Islet Insulin-Positive Cells Using Flow Cytometry Method

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Introduction: Allogeneic bone marrow (BM) in supporting human islet function and survival in vitro has been demonstrated. Whether BM stimulates human islet β cell increase in culture needs to be further confirmed. We proposed that cultured β cell can be accurately quantified with two steps of immuno-staining and flow cytometry method.

Method: Insulin quantification: medium from cultured human islet was harvested and subjected to ELISA assay. Flow cytometry: single insulin positive cells were quantified by LSRII flow cytometry after islets were dissociated by trypsin and collagenases and were fixed and stained with insulin and FITC labeled secondary antibodies.

Result: To avoid insulin loss during the dissociation process, resulting in false negatives, we tested concentrations of trypsin and collagenases and protein release inhibitors of monensin and brefeldin A (BFA). We found that combination of optimal concentration of dissociation enzymes and protein inhibitor significantly increases sensitivity of Flow Cytometry in detecting insulin positive cells. Monensin and BFA with the concentration of 5 μ M and 1 μ M contribute to the optimum detection of insulin positive cells (21% and 18%) compared to untreated controls (9% and 6%). With optimal protocol (0.05% trypsin, 0.2% collagenases and 5 μ M monensin) to evaluate human islet insulin positive cells in islet and islet with BM culture for 39 days, we found that islet with BM culture contains significantly high insulin-positive islet β cells (42.3% \pm 4.5%) compared to islet-only cultures (17.5% \pm 0).

Summary, we have developed a flow cytometry-based analysis method that is capable of quick and accurate quantification of insulin-positive islet β cells in cultured human islets to evaluate effects of BM on human islet β cell regeneration.

Acknowledgement: This work was funded by JDRF 1-2007-180, NIH P20RR018757 (COBRE, PI Dr. Falanga) and Roger Williams Hospital Research fund for Dr. Luo's Research. We appreciate Islet Cell Resource Center (ICR) funded by NIH and JDRF to distribute human islets for this project, and Cell Sorting Core funded by NIH/NCRR 5P20RR018757.

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miRNA BIOMARKERS OF PATHOLOGY AND EXPOSURE IN THE HUMAN PLACENTA

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Problem: Promising links have been examined between environmental exposures, disease progression and altered expression of microRNA (miRNA), small RNAs involved in the post-transcriptional control of gene expression. This project aims to analyze patterns of miRNA expression in the human placenta associated with maternal cigarette smoking (CS) and intrauterine growth restriction (IUGR).

Methods: A placental cell culture model was utilized to investigate effects of nicotine exposure on miRNA expression using a microarray-based approach to identify miRNA whose expression trends were modulated by nicotine exposure. In parallel, human placental samples from IUGR and control infants with or without a history of maternal CS exposure were analyzed by miRNA microarray for aberrant miRNA expression. Those miRNA found to be most differentially expressed are being confirmed using qRT-PCR in independent samples.

Results: In cell culture experiments, nicotine exposure was associated with both up- and down-regulation of specific miRNA, and additional work is confirming these alterations and examining the source of these alterations. Array analysis on human placentas revealed that profiles of miRNA expression differ in growth restricted compared to control infant placentas, and work is ongoing to confirm these findings in an independent series of samples.

Conclusions: Specific miRNA are differentially expressed in response to nicotine exposure, and differ in growth-restricted infants. This suggests a critical role for miRNA in the pathogenesis of infant growth restriction.

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Restored oyster reefs and their impact on the presence of local fauna

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Research Program: U.S. EPA 2008 GRO Fellowship

Problem: The Eastern Oyster (*Crassostrea virginica*) is an ecologically important species that provides multiple ecosystem services, including the potential to increase complex habitat for resident fauna. To this end, the objective of this study was to determine if the presence of restored oyster reefs in Narragansett Bay (RI, USA) increased the abundance and diversity of local macro-invertebrates and finfish.

Methods: In June and July 2009, six baited traps were deployed biweekly one hour \pm of high tide at three oyster reef (OR) and three non-oyster reef (NOR) sites in the Bay. An initial assessment was conducted at the OR sites to determine the density and size-class frequency of the oysters (mean density = 44 ± 18.5 oysters/m², mean size = 43.9 ± 16.5 mm shell height).

Results: There was no significant difference in the abundance of fish and macro-invertebrates between the OR (2.4 fish/trap; 13.3 invert/trap) and NOR (1.3 fish/trap; 11.3 invert/trap) sites. The richness of finfish at OR (0.7 spp./trap) sites was significantly greater than that of the NOR (0.2 spp./trap) sites, but there was no difference in the richness of invertebrates (OR=1.2 spp./trap; NOR=1.1 spp./trap). The catch composition at the OR sites was similar to that of the NOR sites, with Mummichogs and Striped Killifish the most common fish and Green Crabs, Mud Snails and Grass Shrimp the most common invertebrates.

Conclusions: These data suggest that oyster reefs impact the richness of finfish species that occupy a habitat, although they do not necessarily increase the overall abundance of finfish and invertebrates.

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MOLECULAR PATHOLOGY CORE OF THE RHODE ISLAND HOSPITAL COBRE CCRD.

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HIGH THROUGHPUT VALIDATION OF PREDICTED T-CELL EPITOPES IN *F. TULARENSIS*: THE DEVELOPMENT OF A PROTECTIVE T-CELL EPITOPE BASED TULAREMIA VACCINE

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Background: *Francisella tularensis* causes the potentially fatal disease tularemia. Here, we describe our genome-to-vaccine strategy for developing a T-cell epitope-based tularemia vaccine.

Methods: The EpiMatrix epitope-mapping algorithm was used to identify promiscuous T-cell epitopes within the predicted secreted proteins from the *F. tularensis tularensis* genome as well as from known expressed proteins. The top-scoring 40 putative promiscuous class II epitopes were screened in vitro using a recombinant soluble HLA class II competition-binding assay. High affinity epitopes were tested in ELISpot assays using blood from human subjects that had recovered from *F. tularensis tularensis* infection. 14 class II epitope sequences that elicited a robust response in these two assays were incorporated into a DNA vaccine construct and tested in respiratory challenge studies using DRB1*0101 transgenic mice. Mice were immunized with the multi-epitope construct and boosted with epitope peptides formulated in liposomes with CpG oligonucleotides.

Results: Forty-two percent of class II peptides were found to bind soluble DRB1*0101 with high affinity. ELISpot assays showed positive IFN-gamma responses to 21 of 25 individual Class II peptides and to peptide pools, in most human subjects. Splenocytes from vaccinated mice showed higher responsiveness to the vaccine peptides than did splenocytes from sham control mice; these responses were found to correlate with protection: Following respiratory challenge with 5 x LD₅₀ of live, attenuated *F. tularensis holarctica* (LVS), >50% of immunized mice survived while all non-immunized mice died.

Conclusion: Novel T-cell epitopes predicted from the *F. tularensis* genome protect against challenge in a humanized small animal model.

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BAX-induced Cell Death in the Budding Yeast, *Saccharomyces*, Involves Calcium

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Bax is a proapoptotic member of the Bcl-2 family of proteins. Upon activation, Bax binds to the outer mitochondrial membrane, which ultimately induces programmed cell death in mammalian cells. Naturally *Saccharomyces cerevisiae* does not contain BAX; however, when Bax is transformed into the yeast it functions as it does in mammalian cells. We are investigating the role of calcium in Bax-induced cell death in yeast by overexpressing mammalian BAX in our yeast calcium mutants. We have discovered that mutants lacking genes important for calcium regulation including *CCH1*, *CRZ1/TCN1*, *PMC1*, *PMR1* and *VCX1* are all relatively resistant to BAX-induced toxicity. We have observed an increase in BAX-induced toxicity as extracellular concentrations of calcium rise. We are currently working to definitively show that calcium levels change intracellularly by constructing the first yeast cameleon.

Previously, it has been difficult to detect intracellular calcium level changes due to the laborious processes of aequorin testing. This protein complex was taken from jellyfish and is effective in determining calcium concentrations that are in the magnitude of micromolar. Instead of conducting this laborious testing, we can engineer and localize our cameleon to show us intracellular cytoplasmic, endoplasmic reticular and mitochondrial calcium levels. This construct contains two fluorophores which show normal calcium levels (cyan fluorescence) and elevated calcium levels (yellow fluorescence). This in hopes will validate our preliminary calcium data and prove that as intracellular calcium levels rise, so does BAX-induced toxicity leading to apoptosis or programmed cell death.

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Kinetics of T Regulatory Cells in IL-10 null murine pregnancy vs WT Control

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The causes behind many disorders that occur during pregnancy remain enigmatic. Gestational diabetes and pre-eclampsia are two diseases that manifest in women with symptoms primarily during the second and third trimesters of pregnancy. While the causes of these diseases are unknown it is thought that the symptoms that indicate their presence are due to causes that occur at sub-clinical levels in the early stages of pregnancy. T-regulatory cells (Tregs) are known to suppress inflammatory events, and have been particularly researched in the context of auto-immune diseases. While Tregs have not been thoroughly investigated at the maternal-fetal interface, it is widely postulated that Tregs may be intricate regulators of the necessary balance of growth and quiescence that occurs during the orchestration of proper programming of pregnancy. Furthermore, Treg cells are known to be major producers of the immunosuppressive cytokine interleukin-10 (IL-10), thus it is important to discern the availability and activity of Treg cells both in WT and IL-10 null murine based pregnancy contexts. Here, we utilize a mouse model of pregnancy to compare levels of Treg cells at differential stages of gestational age in placental and splenic tissues. Furthermore, we aim to extrapolate from these results the role of Treg cells in murine models of both preeclampsia and gestational diabetes.

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THE EFFECTS OF BETA-GLUCAN TREATMENT ON ENDOTOXEMIA AND SEPSIS

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Problem: Beta-glucans, glucose polymers of the fungal cell wall, stimulate immune cells. The soluble beta-glucan used in this study, IMPRIME-PGG, stimulates immune cells without inducing cytokines. Our aims were 1) to evaluate the effect of beta-glucan pretreatment on the LPS-induced TNF production *in vivo* and 2) to evaluate the effect of beta-glucan on the mortality of and IL-6 production in septic mice induced by cecal ligation and puncture (CLP) when administered after the onset of infection.

Methods: 90 minutes after LPS administration or 6 hours after CLP, serum was isolated. TNF and IL-6 levels were measured by ELISA.

Results: For aim 1, the results indicate that beta-glucan pretreatment for 96 hours prior to LPS stimulation reduced TNF in 4-5-month old male mice, but not in 6-8-week old males. The results also indicate that beta-glucan attenuated TNF in 20-month old female mice, but not in 6-8-week old or 4-5-month old females. For aim 2, the results indicate that beta-glucan given 1 hour after CLP enhanced 10-day survival in female mice and had a moderate enhancement on 3-day survival in males. Beta-glucan did not increase 10-day survival in ovariectomized females or in ovariectomized females given a dose of estrogen, indicating that hormones play a role in the beta-glucan-mediated protection, and that estrogen is necessary, but not sufficient for protection. Beta-glucan reduced IL-6 in male and female mice.

Conclusions: Beta-glucan could be developed as an agent to potentiate immune cell functions of patients without inducing the negative effects of cytokines.

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***In vitro* and *in vivo* evidence for the role of complement activation in preeclampsia**

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Objective: Preeclampsia contributes significantly to maternal and neonatal mortality and morbidity. Delineation of a mechanistic pathway(s) for preeclampsia has remained enigmatic due its heterogeneous etiology. We hypothesize that complement activation is one of the pathways that contributes to the pathophysiology of preeclampsia. Using serum from preeclampsia patients, we address an association between complement activation and preeclampsia.

Methods: Gestational age-matched serum samples from normal pregnancy and preeclampsia patients were used for the study. Serum-induced cytotoxic effects were evaluated on first (HTR-8) and third (TCL-1) trimester trophoblast cell lines and freshly isolated term primary trophoblasts. *In vivo* studies were employed to evaluate preeclampsia serum (PES) to cause hypertension, proteinuria and kidney pathology in pregnant mice. Role of complement was confirmed using multiple approaches. Analysis of complement regulatory proteins, CD55, CD59 and CD46, on trophoblasts was performed by FACS and immunohistochemistry (IHC). Terminal complement complex (TCC) deposition on trophoblast cells was evaluated by immunofluorescence.

Results: PES caused apoptosis in TCL-1, but not in HTR-8 or primary trophoblasts as a result of CD55 and CD59 on latter cells. Heat inactivation, heparin treatment or treatment with C5 and C9 neutralizing antibodies prevented PES-induced TCC deposition and apoptosis of TCL-1 cells. IHC showed reduced CD55 staining in preeclampsia placental tissue compared to normal pregnancy tissue. Both severe and mild PES induced classical symptoms of preeclampsia in pregnant mice with IL-10^{-/-} animals being more sensitive which was reversed by heat inactivation of serum.

Conclusions: Complement activation appears to be one of the mechanisms for placental deficiencies associated with preeclampsia.

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1,25-DIHYDROXY VITAMIN D3 AND NONSTEROIDAL ANTI-INFLAMMATORY AGENTS EXHIBIT ANTI-TUMEROGENIC ACTIVITIES IN HUMAN OVARIAN CANCER CELLS

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Problem: 1,25-Dihydroxyvitamin D3 [1,25(OH)2D3] and nonsteroidal anti-inflammatory drugs (NSAIDS) have been recently shown to inhibit cancer growth in various tissues and hence may be promising “anti cancer agents”. Human ovarian cancer is the most frequently diagnosed malignancy in women. Because of lack of early screening techniques and chemoresistance to drugs, there is a need to explore new preventive approaches for this disease. The objectives of this study were to evaluate the effects of various commonly used NSAIDS [indomethacin (indo), ibuprofen (ibu), naproxen (nap), and acetylsalicylic acid (asa) in human ovarian cancer cell lines, CaOV-3 and OVCAR-3 a) to inhibit cell growth, b) to induce apoptosis, c) to study co-treatment of NSAIDS with 1,25(OH)2D3 to inhibit ovarian cancer cell growth.

Methods: Cell growth was studied by MTS assay, bromodeoxyuridine (BrdU) incorporation assay, and cell cycle analysis. Apoptosis was studied by cell morphological changes, DNA fragmentation analysis and induction of caspase-3 activity.

Results: NSAIDS inhibited the growth of CaOV-3 and OVCAR-3 cells in a dose-dependent manner. Further, NSAIDS caused a block in cell cycle progression, cell morphological changes characteristic of apoptosis, DNA fragmentation and increased caspase-3 activity. These findings indicate that NSAIDS inhibit growth and induced apoptosis by activating caspase-dependent cell death pathways. Further combination of NSAIDS and 1,25(OH)2D3 enhanced ovarian cancer cell growth inhibition.

Conclusions: Our study demonstrates that NSAIDS possess anti-tumerogetic properties in human ovarian cancer cells. These results provide an interesting therapeutic approach for the development of combination treatment based on NSAIDS and 1,25(OH)2D3 for chemoprevention/chemotherapy of ovarian cancer.

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Single and multiple courses of antenatal steroids regulate connexin 36 abundance in the ovine fetal brain

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Problem: Connexin 36 (Cx 36) forms gap junctions (GJ). GJ facilitate intercellular communication and are important in neuronal differentiation during development. Cx 36 is the main GJ between neurons, increases during rodent development and is up regulated by dexamethasone (DEX) in pancreatic islets. The effects of steroids on GJ proteins have not been examined in fetal brain. We examined the effects of single and multiple courses of DEX on Cx 36 abundance in the cerebral cortex (CC), cerebellum (CL) and cervical spinal cord (SP), and differences in Cx 36 protein abundance among fetal brain regions.

Methods: Ovine fetuses at 70% of gestation were examined after a single course of four 6 mg DEX or placebo (PL) injections were given every 12 h for 48 h to the ewes, or the same DEX or PL given course once a wk for 5 wks. Regional brain tissue was frozen after the last DEX or PL dose. Cx 36 protein expression was determined by Western immunoblot.

Results: A single course of DEX increased ($P < 0.05$) Cx 36 protein abundance compared with PL in the CC and SP, and multiple courses increased Cx 36 in SP, but not in CC or CL. Cx 36 was higher in SP than CL.

Conclusions: Maternal DEX treatment regulates Cx 36 proteins *in vivo* in the fetal CC and SP, but not in CL. Different DEX regimens regulate Cx 36 protein abundance in specific brain regions. The greater abundance of Cx 36 protein in SP than CL may suggest the presence of more functional channels in SP in the premature fetal brain.

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CHARACTERIZATION OF THE EXPRESSION OF A PANEL OF CANDIDATE GROWTH REGULATING GENES IN HEPATIC OVAL CELLS

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Type II adult hepatic progenitor cells, often termed oval cells, can be activated to proliferate and differentiate into hepatocytes and bile duct epithelial cells when liver injury is so severe that hepatocyte proliferation is blocked. Experimental models have implicated oval cells as the target of carcinogens and the progenitor of hepatocellular carcinoma. Oval cells express cell surface markers and gene expression characteristics of fetal hepatoblasts and cholangiocytes, but the pathways regulating their growth and proliferation remain unclear. In order to investigate the signaling phenotype of oval cells, we used a panel of candidate growth regulating genes that we had previously identified as differentially expressed in fetal and adult liver. Rats were placed on a modified choline deficient diet plus ethionine injection (CDE) to stimulate the proliferation of oval cells. This protocol resulted in 60-70% of the liver being repopulated by oval cells on day 15. RNA was isolated from fetal, adult, and CDE liver and from a panel of tumorigenic and non-tumorigenic hepatic cell lines. RT-PCR was performed to determine the expression of the panel of candidate growth regulating genes. We found that the hepatic cell lines were heterogeneous in their expression of the panel of genes and did not allow us to distinguish a subset of genes that could be used as biomarkers for a tumorigenic phenotype. However, many of the genes overexpressed in fetal liver were induced in the CDE model, suggesting that oval cells may display a fetal hepatocyte signaling phenotype.

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Enhancing Potency of Anti-HIV Chimeric Immune Receptor (CIR)

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Immunotherapies that have the potential to eradicate reservoirs of HIV infected cells may be useful to suppress or cure HIV/AIDS, in combination with current antiviral drug therapies. T cells expressing chimeric immune receptors (CIR) through gene modification (“designer T cells”) can be redirected to kill HIV infected cells. Previously, an anti-HIV CIR, CD4- ζ (1st generation), was created to bind to HIV-1 gp120 antigen through its CD4 extracellular domain and induction of signaling through cytoplasmic CD3 ζ chain. However, the adoptive transfer of autologous CD4- ζ T cells to HIV/AIDS patients failed to control the viral infection in clinical trials. To improve potency, our laboratory has created 2nd generation anti-HIV designer T cells that incorporate CD28 costimulation (CD4-CD28- ζ). 2nd generation designer T cells kill HIV-Env expressing Jurkat cells and HIV-1 infected CEM-SS cells as well or better than 1st generation designer T cells. Stimulation with anti-CD4 mAb induces more IL-2 production in 2nd generation than 1st generation designer T cells. In addition, 2nd generation designer T cells showed significantly better proliferative response upon culture with HIV-Env expressing HeLa cells or HIV-1 infected CEM-SS cells. These data suggest that gene modified CD8+ T cells could be an effective therapy to eradicate HIV+ cells during and after anti-retroviral therapy. We hypothesize that CD28 domain in the CIR will confer a benefit of longer T cell survival after infusion into HIV patients.

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Identification and Characterization of Lipases Secreted by the Human Pathogen *Leishmania*

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The cell and molecular biology of *Leishmania* are investigated as models of human parasitism. Proteins secreted by the parasite are of interest because through these molecules pathogens are able to sense, respond to, and alter their surrounding environments. For example, lipases are ubiquitous enzymes that hydrolyze the ester linkages of fats to form glycerol and fatty acids. These enzymes are involved in biological activities ranging from cell signaling to nutrient acquisition and have been implicated as virulence factors in some pathogens. We hypothesize that the lipase activity released by *Leishmania* may play an important role in the biology of these primitive parasites.

A PCR-based strategy identified a candidate secretory lipase gene from *Leishmania donovani*. Sequence analysis of LdLIP3 revealed an ORF of 927bp and a deduced protein of 308aa with a predicted molecular mass of 33.0kDa. Southern analysis revealed one copy of this gene in the *Leishmania* genome. RT-PCR showed this gene is expressed in both promastigotes and amastigotes. To further characterize this lipase, an epitope-tagged construct was generated. Western analysis revealed that the expressed protein was secreted by transfected *Leishmania* and lipase activity was determined on purified protein using 4-methylumbelliferyl fatty acid substrates. Taken together, our data supports the hypothesis that a lipase activity encoded by LdLIP3 is secreted by *Leishmania*. This study will serve as the basis for determining the role this enzyme plays in the biology of this important group of human parasitic protozoa.

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The R7 family of GTPase accelerating proteins are upregulated during neuronal differentiation of mouse embryonic stem cells.

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Problem: Regulator of G protein Signaling (RGS) proteins modulate G protein coupled receptor signaling (GPCR) by accelerating the GTPase (GAP) activity of the G β subunits of trimeric G proteins. In addition to their RGS domain the R7 family of RGS proteins, share GGL and DEP domains which have been shown to mediate interactions with a variety of other proteins. RGS6 a member of the R7 family of RGS proteins was recently shown to interact with Slathmin-1 via its GGL domain and accelerate neuronal differentiation of PC12 cells. This effect did not require its GAP activity and suggests that other GGL domain containing RGS proteins may also regulate neuronal differentiation.

Methods: To investigate whether other R7 RGS proteins may be involved in neuronal differentiation, we examined the levels of mRNA for RGS6, 7, and 9 during neuronal differentiation of mouse embryonic stem (mES) cells using reverse transcriptase PCR.

Results: Retinoic acid induced differentiation of mES cells led to a decrease in mRNA expression of the stem cell marker, OCT-4 and an increase in expression of the neuron specific marker MAP2. In addition, differentiation was characterized by an increase in mRNA expression of nestin-1 and PAX-6. Interestingly, RGS6, 7, and 9 mRNA were not detected in undifferentiated cells but were significantly expressed coincident with the detection of nestin-1 mRNA during neuronal differentiation. mRNA for R9AP and R7BP, proteins which regulate the R7 family of RGS proteins paralleled the expression of RGS 6,7 and 9.

Conclusions: The coordinated expression of the R7 family of RGS proteins and the associated proteins, R9AP and R7BP, during neuronal differentiation of mES cells suggests they may play a critical role in neuronal differentiation. Current studies aim to identify potential roles of individual RGS proteins in neuronal differentiation of mES cells using gene knockdown experiments.

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Examination of the Role of Muscle Specific Isoforms of Ufd2a during Myoblast Differentiation

Solares, J, Spinette, S

Ufd2a is an E3/E4 ligase which has three alternatively spliced isoforms, two of which (Ufd2a II & III) are uniquely expressed at different times during the differentiation of skeletal muscle myoblasts. In order to determine the significance of these muscle-specific isoforms, their expression was blocked in a murine myoblast cell line using morpholino oligonucleotides prior to their differentiation in culture. Differentiation was monitored, using immunofluorescence microscopy to observe the expression of the differentiation marker, muscle myosin heavy chain along with DAPI stained nuclei to observe the levels of myoblast fusion. The data obtained thus far has allowed the initial quantification of cell fusion events in murine myoblasts expressing normal levels of each Ufd2a isoform and those in which the muscle specific isoforms have been blocked. Due to various difficulties with the current methods, experiments were performed to optimize the cell staining and adherence to the glass cover slips. Additionally, while it appears that Ufd2a II and III may be required for cell fusion the mechanism of action remains unknown. Since cell cycle exit is required prior to cell fusion we wished to develop protocols to robustly monitor cell cycle exit during differentiation through analysis of p21 expression and DNA content analysis using Flow Cytometry..

In order to verify that the phenotype is specifically due to a lack of Ufd2a II and III expression, cells were transfected with exogenous human Ufd2a III tagged with GFP after blocking the endogenous muscle isoforms with morpholinos. The expression of the exogenous forms were viewed using fluorescence microscopy throughout the differentiation process. These experiments showed that transient expression lasted only 48 hours while full differentiation required 3-4 days. Therefore, a stable line of cells expressing the GFP-tagged Ufd2aIII is being developed.

Defining the role of Ufd2a in myoblast fusion may contribute important information for developing future methods of muscle regeneration for patients with injuries, or muscular dystrophy since myoblast fusion is an early essential step in muscle repair.

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“Cytokine-induced dopaminergic differentiation of the immortalized mesencephalic cell line CSM14.1 in vitro.”

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Problem: The potential clinical impact of cell replacement in neurological degenerative diseases has already been shown by transplantation of fetal mesencephalic cells into patients with Parkinson`s disease. However ethical issues and the limited availability of human fetal tissue constrict a general application. Immortalized mesencephalic cell lines may be a suitable alternative. We cultured CSM14.1 neural progenitor cells which were established via retroviral transduction of the temperature-sensitive mutant of the SV40 Large T Antigen into primary cells derived from mesencephalon of rats of embryonic day 14. Previously it was shown that these cells in vitro express Nurr1 followed by time-dependent up-regulation of the enzymes tyrosine hydroxylase (TH) und aldehyde dehydrogenase 2 under defined conditions (cultured at 39°C in DMEM containing 1%FCS). The present study examined the influence of the cytokines interleukin-1 (IL1), interleukin-11 (IL11), leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF) on CSM14.1 cells in vitro. CSM14.1 cells cultured at defined conditions were compared to those cultured at the same conditions with addition of cytokines for 14 days.

Methods: By using histology, immunocytochemistry, and Western blotting to determine the presence of neural, glial and dopaminergic differentiation, we demonstrate that in response to cytokines after 14 days the cells strongly express characteristics of dopaminergic neurons in comparison with a CSM14.1 cells cultured without cytokines.

Results: Stereologic analysis revealed that in cultures exposed to cytokines cell somata appeared multipolar, the formed larger processes ($p = 0.02$, U-Test) and created a connective network. In Western blotting we observed an increase of neural markers NeuN and NSE. The protein levels of dopaminergic markers Nurr1 and TH were barely detectable in both culture, with and without cytokines. But further increase in Nurr1 and TH expression in cultures exposed to cytokines mixture was not observed.

Conclusion: These data suggest that cytokines support the conversion of progenitor cells into dopaminergic neurons.

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Screening Compounds for Ligand Activity with Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR)

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Problem: Constitutive Androstane Receptor (CAR) and Pregnane X Receptor (PXR) are closely related nuclear receptor proteins that share some ligands and target overlapping sets of genes, influencing all phases of drug metabolism. There is a need to define structure-activity relationships among CAR and PXR ligands in order to optimize drug therapies. Compounds with demonstrated unique activity on any one receptor are potentially valuable diagnostic tools for future *in vivo* molecular targets.

Methods: Sixty-one compounds, encompassing 7 core chemical structures, underwent cell-based ligand activity screening in human hepatoma cell line HuH7. Expression plasmids for PXR and 2 biologically relevant CAR variants-- CAR1 (constitutive activity) and CAR3 (inducible activity) --were co-transfected with Luciferase reporter plasmids under the control of CAR or PXR consensus regulatory elements. Luciferase activity was measured and used to define ligand binding, agonist, antagonist and inverse agonist activity.

Results: Twenty-eight compounds were ligands of CAR1, CAR3 and PXR, 13 were ligands of CAR only and 5 were PXR ligands only. One compound was toxic and 14 did not modulate any receptor activities. There were distinct differences in agonist vs. inverse agonist/antagonist effects associated with structurally different compounds.

Conclusions: This work provides evidence for new CAR ligands, some of which have splice-variant-specific effects. These results are the basis for protein modeling and docking experiments to elucidate the subtle differences in CAR-ligand and non-ligand activity within structurally similar chemical groups and to help predict agonist, antagonist or inverse agonist activity. (Supported by RI-INBRE Grant # P2ORR016457 from NCCR, NIH)

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STRUCTURE ACTIVITY RELATIONSHIPS OF PYRETHROID INSECTICIDES ON THE HUMAN T-TYPE VOLTAGE-SENSITIVE CALCIUM CHANNEL (Ca_v3.2)

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Problem: Pyrethroids are widely used insecticides in both agricultural and vector control programs. Given the widespread use of pyrethroids for the control of insect vectors of devastating human and animal diseases (particularly in urban environments) and additional exposure via dietary uptake, human consumption is virtually assured. The purpose of this research was to determine the effects of pyrethroid insecticides as a class on the current characteristics of a human t-type voltage-sensitive calcium channel (Ca_v3.2).

Methods: Human Ca_v3.2 cDNA was transcribed into cRNA using the mMessage mMachine *in vitro* transcription kit and injected into defolliculated *Xenopus* oocytes. Human Ca_v3.2 currents were electrophysiologically characterized using the two-electrode voltage clamp technique with Ba²⁺ as a charge carrier. Dose response curves were generated and relative indices of potency (EC₅₀) and efficacy (E_{max}) were obtained. Pyrethroids effects on the peak current were determined following perfusion with various concentrations of deltamethrin, α -cyhalothrin, bifenthrin, fenvalerate, esfenvalerate, fenpropathrin, permethrin, tefluthrin, bioallethrin, and α -cyfluthrin.

Results: Dose response curves were obtained for deltamethrin, α -cyhalothrin, bifenthrin, fenvalerate, esfenvalerate, fenpropathrin, permethrin, tefluthrin, bioallethrin, and α -cyfluthrin. and relative indices of potency and efficacy extrapolated. Overall, pyrethroids as a class did not modify Ca_v3.2 in a consistent manner. Pyrethroids that possess an α -cyano moiety were more potent and efficacious than the ones that lack this moiety. Moreover, the presence of a halogenated site of unsaturation in the acid portion of the molecule appears to also play a role in the mechanism of action of these compounds on this channel.

Conclusions: Pyrethroids are potent and stereospecific inhibitors of the human t-type voltage-sensitive calcium channel (Ca_v3.2). These compounds, as a class do not modulate Ca_v3.2 in a consistent manner.

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INHIBITION OF *PSEUDOMONAS AERUGINOSA* AND *STAPHYLOCOCCUS AUREUS* GROWTH ON NANOROUGH POLYVINYL CHLORIDE ENDOTRACHEAL TUBES

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Problem: Ventilator associated pneumonia (VAP) is a nosocomial infection resulting in significant morbidity and mortality. Endotracheal tubes (ETTs) that can resist infection are available, however none have focused on altering the polymer surface. Growth of *Pseudomonas aeruginosa* (*P. aeruginosa*) and *Staphylococcus aureus* (*S. aureus*), was compared between conventional (control or nano-smooth) and nano-rough (<100 nanometer featured) polyvinyl chloride (PVC) ETTs.

Methods: A lipase 0.1% solution in potassium phosphate was used to soak ETTs with *Rhizopus arrhizus* (Nano R) or *Candida cylindracea* (Nano C) for 48 hours to change nano-smooth to nano-rough ETTs. The surface was evaluated using scanning electron microscopy and water contact angle. After sterilizing, either *P. aeruginosa* or *S. aureus* was inoculated to control and nano-rough ETTs, then they were incubated overnight at 37°C. Bacteria were collected after 4, 12, 24 and 72 hours, and bacterial growth was measured using optical density and analyzed using analysis of co-variance.

Results: Nanoscale features on PVC were successfully created. Untreated ETT had higher water contact angle compared to nano-rough ETT. Both *P. aeruginosa* and *S. aureus* grew up to 72 hours after inoculation. At 24 hour, *P. aeruginosa* growth was significantly lower on Nano-C and Nano-R compared to control ETTs, however no difference in other time points. Compared to controls, *S. aureus* growth was significantly lower on Nano-C and Nano-R at all time points.

Conclusions: Growth of *P. aeruginosa* and *S. aureus* on nano-rough ETTs was inhibited. This nanotechnology for modifying ETT polymer is promising to reduce VAP *in vivo*.

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De-Immunization of Human Factor VIII: Identification of Epitopes in the C2 Domain

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Problem: Hemophilia A patients are prone to develop inhibitory immune responses to the very treatment they require. Since their bodies do not produce fVIII in adequate supply, therapeutic administration of the protein is interpreted by their immune systems as foreign.

Methods: Using an epitope-mapping algorithm called EpiMatrix, we have identified sites of potential immunogenicity within fVIII protein. By performing targeted amino acid modifications on these putative epitope clusters, it is possible to reduce their HLA binding propensity without altering the functional ability of fVIII to initiate clotting. We have begun this process with the C2 domain of fVIII because it not only is a major target for “inhibitors”, but it also contains immunodominant epitopes identified by T-cell responses in both mice and in patients with hemophilia A. Using EpiMatrix, we selected 10 peptides in human fVIII that would be predicted to bind to eight class II HLA DR molecules that encompass over 95% of the World population. Six of the ten were confirmed by in vitro binding assays to bind to HLA at $IC_{50} < 100\mu M$.

Results: Immunization of fVIII knockout mice with these peptides or fVIII protein in CFA demonstrated immunogenicity. We report here that modification of 1-2 residues significantly reduced the immunogenicity of several of these peptides in thymidine incorporation assays.

Conclusions: These studies are currently being confirmed in HLA-DR transgenic mice but they provide evidence that a recombinant fVIII product may be produced that is of reduced immunogenicity for treatment of hemophilia A patients. (Supported by NIH R43 HL088834-01)

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DEVELOPMENT OF NOVEL BISPECIFIC ANTIBODIES FOR FACILITATING SITE-SPECIFIC TISSUE REPAIR

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Problem: The wound environment remains a perpetual target for improving the speed, sterility and security of tissue repair. We hypothesize that Bispecific Antibodies (BiAbs) will allow direct recruitment and trafficking of effector cell populations, such as stem/progenitor cells or macrophages, to sites of injury where they can facilitate tissue repair and regeneration.

Methods: BiAbs were constructed by chemically heteroconjugating one monoclonal antibody (mAb) specific for a target cell population (*i.e.* F4/80 expressed by macrophages, or c-kit expressed by stem cells) with a second mAb directed at antigens upregulated at the site of injury (*i.e.* VCAM or Fibrinopeptide A; FibA). Efficiency of heteroconjugation was measured by SDS-PAGE densitometric analysis. Specific BiAb binding to effector cell target populations was observed by flow cytometry. The functional ability of BiAbs to mediate binding of a specific cell population to a specific antigen was observed and quantified in a novel biological assay.

Results: The composition of final products was determined to be 66.75%, 58.76%, and 24.80% active heteroconjugates of F4/80x FibA, F4/80xVCAM, and cKitxVCAM, respectively. BiAb to effector cell binding was observed up to 71.7%. Effector cells armed with BiAb bound their targets up to 4 times more frequently than unarmed controls *in vitro*.

Conclusions: Having successfully developed and validated *in vitro* three distinct BiAbs, we will use them to study the trafficking of targeted cell populations to injured tissue in two murine models of wound repair. Finally, we will measure effects of BiAb-mediated targeting of specific cell populations to these injured tissues on tissue repair and function.

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G protein coupled receptor kinase independent desensitization of the D2 Dopamine receptor by Arrestin3.

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Problem: Dopamine is one of the most important neurotransmitters in the brain. There are 2 main classes of dopamine receptors D1-like (D1 and D5) and D2-like (D2, D3 and D4). Antagonism of the D2-class of dopamine receptors (D2R) is highly correlated with the therapeutic effect of antipsychotic drugs. This may suggest that the disease (schizophrenia) may arise from aberrant dopamine signaling. One important mechanism by which receptor activity, localization, and expression level are controlled is through regulation by G-protein coupled receptor kinase (GRK) and arrestin. The objective of this study is to determine the role of GRK and arrestin in the desensitization of the D2R.

Methods: The Delta Opioid receptor (DOR) and D2R activated inwardly rectifying potassium channel (KIR3) responses were measured in *Xenopus* oocytes using two-electrode voltage clamp electrophysiology. Desensitization of receptor-activated responses was measured in oocytes expressing the receptor and channel alone or with GRK3 and/or arr3.

Results: As previously described, both GRK3 and arr3 co-expression with the DOR was required to mediate significant receptor desensitization. In contrast, arr3 expression alone produced significant desensitization of the D2R.

Conclusions: These data suggest a novel phosphorylation independent mechanism by which D2R can activate and recruit arrestin. Current investigation includes determining the residues on D2R responsible for arrestin activation and determining whether they are transposable to other GPCRs.

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TEMPORALLY CONTROLLED CARDIAC-SPECIFIC ACTIVATION OF PI3K α - A NOVEL CONDITIONAL TRANSGENIC MOUSE MODEL

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Problem: Cardiac PI3K signaling plays important roles in cardiac growth and is highly regulated during development with highest levels found during the fetal-neonatal transition period and lowest in the adult. Prolonged constitutive activation of PI3K α in the heart results in hypertrophy, making the study of the roles of PI3K in cardiac growth difficult.

Methods: To examine the impact of temporal overexpression of PI3K α in the heart, we have engineered a tetracycline transactivator (tet-off) controlled inducible transgenic mouse model for cardiac-specific overexpression of PI3K α .

Results: Cardiac PI3K activity and Akt phosphorylation were significantly increased in adult mice after transgene induction following removal of doxycycline for 2 weeks. Heart weight to body weight ratio was not changed and there were no signs of cardiomyopathy. Overexpression of PI3K α resulted in increased left ventricular (LV) developed pressure, LV dP/dt max and LV dP/dt min, but not heart rate, as assessed in Langendorff hearts. Mice overexpressing PI3K α also had increases in the levels of Ca²⁺-regulating proteins, including the L-type Ca²⁺ channels, ryanodine receptors, and SERCA2a.

Conclusion: Temporally controlled overexpression of cardiac PI3K α does not induce hypertrophy or cardiomyopathy but results in increased contractility, probably via increased expression of multiple Ca²⁺-regulating proteins. These distinct phenotypes suggest a fundamental difference between transgenic mice with temporal or prolonged activation of cardiac PI3K α .

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VACCINIA CHALLENGE WITHOUT B CELL PRIMING

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Problem: We hypothesized a vaccine based on vaccinia/variola cross-reactive class II T-cell epitopes (“VennVax”), identified using bioinformatics and immunological methods, would protect against vaccinia challenge in HLA transgenic mice.

Methods: Using vaccine design algorithms, EpiVax developed two multi-epitope genes, containing 25 HLA class II T-cell epitopes. DRB1*0301 transgenic mice were intramuscularly immunized twice with DNA vaccine constructs and boosted intranasally twice with epitopes formulated as peptides in liposomes with CpG oligodeoxynucleotide. Vaccine immunogenicity was measured prior to vaccinia challenge by IFN γ ELISpot, multiplex cytokine bead array and thymidine incorporation in peptide-stimulated splenocyte cultures. Mice were challenged with 10X LD₅₀ vaccinia WR, measuring weight change and survival post-infection. Vaccinia- and vaccine-specific antibody levels pre/post-challenge were measured by ELISA.

Results: Immunization of mice stimulated significant T cell responses to 6/10 peptide pools by IFN γ ELISpot. Pooled epitopes stimulating >50 SFC/10⁶ splenocytes compared to non-immunized mice were considered immunogenic (N=3 per group, p<0.001). Significant peptide-stimulated proliferation was observed for 8/10 pools and IFN γ , IL-2 and MIP-1 α production for various pools (p<0.05). 100% of vaccinated mice (N=18) survived vaccinia challenge, showing no signs of illness, while only 19% of control mice recovered after significant weight loss (p<0.001). Vaccination elicited no significant antibody response pre-challenge.

Conclusions: VennVax provides excellent protection against lethal challenge in a humanized animal model with no B cell component to vaccine immunogenicity, suggesting T cell-oriented protection. VennVax priming before licensed live-attenuated vaccination such as with MVA would be a dose-sparing strategy to expand the supply of smallpox vaccine.

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Using Functional Genomics to Discover Genes that Confer Resistance to Indolmycin, a Potential Antibacterial Drug

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Problem: Aminoacyl-tRNA synthetase inhibitors are a promising class of antibacterial drugs. Indolmycin, produced by *Streptomyces griseus* ATCC 12648, is a competitive inhibitor of tryptophanyl-tRNA synthetase that has attracted much attention as a potential antibacterial agent. In the interest of predicting the clinical efficacy of this antibiotic, we sought to discover genes in bacterial populations that mediate resistance to indolmycin. The purpose of this study was to discover indolmycin resistance genes and to determine their distribution in bacterial genomes.

Methods: Bioinformatic analyses of the genomes of indolmycin-resistant *Streptomyces* bacteria led to the discovery of several auxiliary tryptophanyl-tRNA synthetases. These putative indolmycin resistance genes were functionally characterized by cloning and heterologous expression in an indolmycin-sensitive strain. Reverse transcription polymerase chain reaction was employed to analyze the transcription profiles of the resistance genes.

Results: Several antibiotic-resistant, auxiliary tryptophanyl-tRNA synthetases were discovered in *Streptomyces* bacteria. The synthetases were divided into two distinct classes based on their sequences. Homologs of these synthetases were also identified in several bacterial pathogens (e.g., *Klebsiella pneumoniae*, *Salmonella typhimurium*, *Enterobacter cancerogenus*). The transcription of one class of synthetase was induced by indolmycin, while the other class was constitutively transcribed.

Conclusions: Resistance to indolmycin is common in the *Streptomyces* genus. Based on the wide distribution of homologs of the indolmycin resistance genes in many bacterial genera, we predict that resistance to this antibacterial agent is widespread. This study illustrates how bioinformatic analyses coupled with functional studies can be used to identify novel resistance genes and to predict resistance profiles.

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COMPARISON OF EFFICACY OF PREFABRICATION OF THE ISCHEMIC FLAPS WITH AAV2-MEDIATED VEGF AND bFGF GENE THERAPY

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Problems: In reconstructive surgery, necrosis of transferred flaps is a major problem often resulting in a hard-to-treat wound. Gene therapy approaches have been attempted experimentally to combat this problem. We evaluated the efficacy of prefabricating ischemic flaps with AAV2-medicated VEGF or bFGF gene transfers in promoting survival.

Methods: Twenty-seven Sprague-Dawley rats were divided (7 to 10 rats per group) into two experimental groups and one non-treated control group. 2.9×10^{10} AAV2-VEGF or 7.5×10^{10} AAV2-bFGF viral particles were injected into the dorsum of each of the rats of the two experimental groups. Two weeks post-injection, a 3 x 10 cm flap was raised from the injection area. In the control group, this flap was raised with prior prefabrication. One week after post-surgery, flap viability was measured. Immunohistochemical staining of VEGF or bFGF were evaluated.

Results: Flap viability was significantly improved by the AAV2-VEGF prefabrication ($p < 0.01$). The greatest survival area among all samples was in a flap prefabricated with AAV2-bFGF; however, the mean viability was equivalent in two groups. Despite this similarity, the flap viability of the AAV2-bFGF group was not statistically different from that of the non-treatment group, because of a wide standard deviation. Immunohistochemical staining showed greatly enhanced VEGF or bFGF expression in treated groups.

Conclusions: Ischemic flap prefabrication with AAV2-VEGF more consistently improves flap survival as compared to prefabrication with AAV2-bFGF. Prefabrication with AAV2-bFGF both effectively increases flap vascularity and also increases mean survival areas, but it does not lead to constant improvement in flap survival.

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THE ROLE OF GINSENOSEIDE RG1 IN AUGMENTING SURVIVAL OF THE ISCHEMIC SKIN FLAP: IN VITRO STUDIES OF CELL PROLIFERATION AND TUBE FORMATION AND IN VIVO EFFECTS

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Problems: A central event underlying wound-healing processes is angiogenesis, to which cellular proliferation and capillary tube formation are imperative. Ginsenoside Rg₁, an important constituent of ginseng, is a promoter of angiogenesis. However, the role of Rg₁ in promoting skin flap survival has not been investigated.

Methods: In a culture model, we first tested the efficacy of Rg₁ in promoting proliferation and capillary tube formation of HUVECs. Cell proliferation was assessed with MTS assay, and tube formation was recorded under a confocal microscope. Secondly, we studied the proliferation of cultured rat skin fibroblasts using an automated, live-cell imaging platform (IncuCyte) obtained over a week period. Finally, to assess effectiveness of Rg₁ in vivo, 20 Sprague-Dawley rats were divided into 2 groups. 2.1ml of saline containing 0.2 mg of Rg₁ was injected into flap territory and the flap was raised. In the control group, the flap was raised without preceding injections. One week later, flap viability was measured. Histologic sections were stained immunohistochemically.

Results: Rg₁ significantly enhanced the proliferation of HUVECs and promoted capillary tube formation. Addition of Rg₁ significantly improved closure rates of the “wounds” in the monolayer of fibroblasts. In the rat model, flap viability was significantly improved with Rg₁ injection compared to the control (p<0.05). Enhanced von Willebrand factor expression was found after injection.

Conclusions: Use of Rg₁ significantly improves proliferation of skin fibroblasts and increases ischemic flap survival. Rg₁ may induce the production of factors pertinent to angiogenesis, thus promoting angiogenesis and augmenting healing in the ischemic flap.

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DE-TOLERIZATION OF ANTI-DEC-205 FOR HIV SUBUNIT VACCINE DELIVERY

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Problem: Co-administration of non-specific dendritic cell maturation factors and anti-DEC-205 antibody stimulates strong inflammatory immune responses. Without such factors, anti-DEC-205 induces antigen-specific tolerance rather than immunity. We hypothesize that regulatory T-cell epitopes contained in anti-DEC-205 promote a tolerogenic response that is only overcome through co-administration of non-specific immuno-stimulators. We have verified experimentally that these epitopes generate an expansion of regulatory T cells and suppress inflammatory immune responses. Here, we begin a proof-of-principle demonstration that a pro-inflammatory and non-tolerogenic anti-DEC-205 antibody can be developed.

Methods: We screened the anti-DEC-205 sequence computationally for putative HLA DR4-restricted, regulatory T-cell epitopes as targets for mutations that will reduce epitope binding affinity for HLA. Amino acid substitutions predicted to interfere with HLA binding were identified and experimentally verified in HLA DR4 binding assays. Sequence modifications were incorporated into an array of anti-DEC-205 antibody variants recombinantly fused to a test antigen, HIV Gag, to be produced in a mammalian expression system.

Results: We chose to produce six variant antibodies, containing up to three amino acid substitutions, one or two for each of the four epitopes with high DR4 affinity. These variant antibodies that do not interfere with dendritic-cell targeting are being evaluated for reduced tolerogenicity, as well as for enhanced Gag immunogenicity, in terms of cellular and humoral responses.

Conclusions: We predict that the modification of regulatory T-cell epitopes will significantly diminish tolerogenicity, enabling the use of modified anti-DEC-205 as a HIV antigen-delivery system that obviates the dangers associated with non-specific activation of the immune system.

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In Situ Nanotechnology Sensors for Improving Implant Success

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Problem: Titanium (Ti) is the most widely used material for orthopedic implantation, such as for total hip replacements. However, for a variety of reasons, current bone implants do not effectively serve patients for their entire lifetimes. Using nanotechnology, in this study, Ti was modified into an electrochemical electrode to determine what type of tissue (if any) is growing surrounding a hip implant.

Methods: For this purpose, Ti substrates were anodized with diluted 1.5% (by weight) hydrofluoric acid in order to form a nanoporous surface on Ti, and then were coated with cobalt nitrate as a catalyst for carbon nanotube growth. Afterward, multi-walled carbon nanotubes (MWCNTs) were grown by chemical vapor deposition (CVD) on the nanopores of the anodized Ti, called "MWCNTs-Ti". MWCNTs were necessary to electrically measure the type of tissue growing next to the implant. The electrodes were further modified to release drugs on-demand upon the application of a small voltage.

Results: The results of this studying demonstrated the possibility of transforming currently implanted Ti into an electrode which can not only send what type of tissue is growing next to an implant, but also release drugs on-demand to promote bone growth as well as inhibit scar tissue and biofilm formation.

Conclusions: Such sensors are also currently being explored to transmit information from the surface of the implant to a hand-held device to further aid in the clinical diagnosis of implants.

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EXPRESSION OF THE GLUCOCORTICOID RECEPTOR IN RENAL CELL NEOPLASMS: DIAGNOSTIC, HISTOGENETIC, AND PROGNOSTIC IMPLICATIONS.

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Background: Glucocorticoids (GCs) are steroid hormones involved in a variety of physiologic and pathologic processes, such as cellular differentiation, growth, inflammation, and the immune response. GCs mediate their effect by binding to the glucocorticoid receptors (GRs), members of the steroid hormone receptor superfamily, which are expressed in a variety of target tissues, including the kidney. Our goal was to determine the expression pattern and prognostic significance of GR in renal cell neoplasms (RCNs).

Design: Paraffin embedded microarray specimens from 187 consecutive patients with RCNs were analyzed for GR expression by IHC. A rabbit polyclonal Ab PA1-511A from Affinity Bioreagents was used as the primary Ab. Cases were stratified into 132 clear cell renal cell carcinomas (CRCC), 25 papillary RCC (PRCC), 16 chromophobe RCC (CHRCC), and 14 oncocytomas (OC). The intensity of protein expression was scored semiquantitatively on a scale of 0-3+.

Results: Strong nuclear GR expression was present in normal renal glomeruli and in the proximal convoluted tubules, whereas distal convoluted tubules and collecting ducts were negative. In the RCNs the staining pattern was similar to that in normal kidney with predominant nuclear GR localization. GR expression was found in the majority of CRCC (68%), in 24% of PRCC, and in only 6% of CHRCC and 14% of OC ($P<0.005$). Within the CRCC group the vast majority of positive cases (88%) demonstrated strong nuclear reactivity (2+ and 3+), whereas none of the CHRCCs and OCs showed strong expression. Univariate analysis revealed a significant direct correlation between GR expression and overall survival in the CRCC group ($P=0.03$). By the end of follow-up 88% of CRCC patients with positive GR staining were alive as opposed to 59% of the patients whose tumors were negative. Multivariate analysis indicated that GR expression was an independent predictor of survival ($P=0.04$).

Conclusions: The majority of CRCC strongly express GR, distinguishing them from CHRCC and OC. This expression pattern reflects the histogenetic origin of CRCC from the proximal nephron. GR may be considered in the IHC panel to more accurately subtype RCNs. Moreover, GR expression proved to be a strong predictor of patient survival in CRCC.

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AMBIENT HIGH GLUCOSE ENHANCES PROLIFERATION AND EXTRACELLULAR MATRIX PRODUCTION OF RENAL PROXIMAL TUBULAR CELLS BY SUSTAINING EXPRESSION OF EPIDERMAL GROWTH FACTOR RECEPTOR

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We examined the mechanisms underlying the effect of high ambient glucose on renal tubular epithelial cells (PTEC) using immortalized human PTEC and HK-2 cells. High glucose (450mg/dL) sustained epidermal growth factor receptor (EGF-R) expression in PTEC which was cultured for longer than 120 hours. Sensitivity to exogenous EGF was significantly higher in PTEC under high glucose environment than under normal ambient glucose (100mg/dL). Decreased transcription of EGFR was shown by RT-PCR in PTEC with high glucose. Fluorescent labeling study suggested delayed degradation of EGFR in PTEC with high glucose. Cell proliferation, type IV collagen synthesis and the levels of phosphorylated ERK were increased in PTEC cultured under high glucose condition. These effects of high glucose were partly attenuated by suppressing EGFR expression by transfection of short hairpin (sh) RNA against EGFR. Over expression of wild type EGFR elevated basal levels of proliferation, ECM production and ERK phosphorylation in PTEC and decreased the impact of ambient high glucose on these parameters. Among five cognate ligands for EGFR [TGF- β , amphiregulin (AREG), betacellulin (BTC), heparin-binding EGF, epiregulin and neuregulin), HK-2 cell expressed AREG and BTC constitutively. Co-transfection of shRNA against AREG and BTC induced significant decreases in proliferation and ECM production and abrogated the effects of high glucose on PTEC. These results suggest that ambient high glucose promotes cell proliferation and ECM production in PTEC by sustaining the expression and effects of EGFR. Endogenous AREG, BTC and EGFR signaling through ERK activation may play a pivotal role in this system. (NIH P20 RR018728)

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INTERACTION OF MATRILIN3 (MATN3) WITH CHONDROGENIC GROWTH FACTOR TGF- β 1 IS ENHANCED BY DECORIN.

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Problem: The absence of extracellular matrix protein MATN3 in mice leads to mis-regulation of chondrocyte differentiation in the developing epiphyseal growth plate, and early onset of OA. Recently, we showed MATN3 increased cell proliferation and chondrogenesis of synovial fibroblasts treated with TGF β 1. However mechanism for MATN3's regulatory function is unknown. The aim of this study is to characterize the direct binding interaction of MATN3 with TGF β 1, and further examined whether decorin, a proteoglycan that strongly interacts with TGF β 1 competes binding with MATN3.

Methods: Initially, MATN3 was coated on to 96 well plates, and non-specific binding sites were blocked. The amount of TGF β 1 bound to MATN3 was measured using ELISA based assay. In addition, the binding of TGF β 1 to MATN3 in the presence of decorin was also examined. These results were further confirmed with surface plasmon resonance (SPR) analysis, where TGF β 1 (6.25-200nM) or in combination with decorin was injected at 15ul/min over a CM5 sensor chip immobilized with MATN3. Scatchard analysis of the SPR data was used to calculate binding affinity of TGF- β 1 to MATN3.

Results: The saturation of TGF β 1 binding to MATN3 indicates that they have a strong biochemical interaction. The high binding affinities 11nM and 128nM indicate that there are two possible TGF β 1 binding sites on MATN3. Furthermore, decorin increased the binding of TGF β 1 to MATN3 by acting as a linker protein between TGF β 1 and MATN3.

Conclusion: The findings suggest MATN3 may regulate cellular changes by directly, and indirectly binding via decorin to chondrogenic growth factor TGF β 1.

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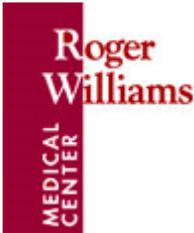
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