Robert Wood Johnson Medical School 2005

MD/PhD Program Symposium
2005
Robert Wood Johnson Medical School

MD/PhD Program Symposium

Tuesday July 19, 2005
Dean's Conference Room
Piscataway, New Jersey
Program

Continental Breakfast 8:30 to 9:00 am

Introductory Remarks 9:00 to 9:30 am

Harold L. Paz, M.D.
Dean, Robert Wood Johnson Medical School

Terri Goss Kinzy Ph.D.
Assistant Dean for Medical Scientist Training and M.D. Ph.D. Program Director, Robert Wood Johnson Medical School

Student Presentations (Session 1) 9:30 to 11:00 am

Break 11:00 to 11:15 am

Student Presentations (Session 2) 11:15am to 12:30 pm

Luncheon 12:30 to 2:00 pm

Keynote Address

William Hait, M.D., Ph.D.
Director, Cancer Institute of New Jersey
Associate Dean for Oncology Programs, Robert Wood Johnson Medical School
Acknowledgments

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In addition, we would like to thank Janice Shanahan, Christopher M. Stastny and Perry Dominguez for their support in planning and organizing this event.

The UMDNJ – Robert Wood Johnson Medical School MD/PhD Program is a collaboration with Rutgers, the State University of New Jersey and Princeton University.
Student Presentations (Session 1)

Dr. Nicola Partidge, Chair, Department of Physiology, UMDNJ-Robert Wood Johnson Medical School (moderator)

Jean-Paul Abboud, “An In-Silico Model of Mouse Retinal Neurogenesis that Accounts for Both Cell Number and Cell Class.”

Brian K. Barlow, “A Fetal Risk Factor for Parkinson’s Disease.”

Parima Daroui, “Topoisomerase I-mediated DNA Damage Induced by Oxidative Stress.”

Bonnie Huang Hall, “Image-Guided Decision Support in Diagnostic Pathology.”

Denise L. Livingston, “Regional and Gender Specific Effects of Estradiol on Astrocytes.”

Akiva J. Marcus, “Adult Bone Marrow Stromal Cells in the Embryonic Brain: Long-Term Survival and Sub-region Specific Differentiation.”


Break 11:00 – 11:15

Student Presentations (Session 2)

Dr. Lori Covey, Associate Professor, Department of Cell Biology and Neuroscience, Rutgers, The State University of New Jersey (moderator)

Marcelo Rocha, “Na+/H+ Exchanger Inhibition Protects Cultured Rat Mesencephalic Neurons Against Malonate Toxicity.”

Ian Rossman, “En2, an Autism-Associated Gene, Regulates Cerebellar Granule Neuron Precursor Responses to Extracellular Signaling Molecules During Development.”

Abhishek Singh, “Tropomyosin has a Co-Requirement of Coiled Coil Flexibility and Specific Non-Interface Residues for Actin Binding.”

Natasha Telesford, “Integration of Mitochondrial on DNA in Yeast.”

Stacy Trooskin, “Racial Disparities in Hepatitis C. Testing.”

Tanisha A. Williams, “Polymorphic Alleles of Genes Affecting Innate Immunity, Antioxidant Mechanisms and Neurodevelopment: The Gene-Teratogen Model as Applied to Autistic Disorder.”
Abstracts
An In-Silico Model of Mouse Retinal Neurogenesis that Accounts for Both Cell Number and Cell Class.

Abboud, J. P. (1), (2); Hayes, N. L. (2); Nowakowski, R. S. (1), (2)

(1) Biomed. Eng., Rutgers University, Piscataway, NJ

(2) Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ

The neurons and glia of the mouse retina are produced from embryonic day 10 (E10) to postnatal day 11 (P11). At E10 the retina consists solely of proliferating cells whereas at P11 there is ~150-fold increase in the number of cells, all of which are non-proliferating. During this period there are systematic changes in the proportion of daughter cells that continue to proliferate vs those that quit (Q) the cell cycle to become postmitotic and in the classes of cells produced. We developed an in silico model of retinal neurogenesis based on: 1) an estimate of 22 cell cycles during this period, 2) changes in Q as a function of elapsed cell cycle, and 3) a set of probability density functions that describe the cell fate per cell cycle for each of the retinal cell classes. Comparison of the model output with data from actual retinal development established that a sigmoidal-shaped pathway describing cell cycle exit from Q=0 to Q=1 and modified normal distributions for each cell class probability density function accounts well for both the number and classes of cells produced both in toto and over the developmental period. The power of this computational model is its predictive capability. This can be exploited by systematically perturbing the model to develop quantitative hypotheses about the difference in cell composition between central and peripheral retina and the variability occurring subsequent to aberrant development, e.g., in albinism, ocular albinism and various mutants and knockout. Preliminary findings show that cell cycle exit decisions alone can produce changes in the ratio of neuronal cell classes produced. Cell fate determination can also produce changes in cell class composition as can a combination of the two. Specific predictions of the time and magnitude of differences in development can be made for subsequent experimental validation.
A Fetal Risk Factor for Parkinson’s Disease.

Barlow, B. K.; Richfield, E. K.; Cory-Slechta, D. A.; Thiruchelvam, M.

Environmental and Occupational Health Sciences Institute, UMDNJ–Robert Wood Johnson Medical School, Piscataway, NJ

Idiopathic Parkinson’s disease (PD) is a neurodegenerative disorder that clinically emerges after a threshold loss of nigrostriatal dopaminergic (DAergic) function has occurred, typically affecting individuals later-in-life. Environmental insults, such as exposure to agrichemicals, have been identified as risk factors for PD based on clinical case reports and epidemiological findings, with support from animal models. It has remained unclear, however, whether insults leading to idiopathic PD occur during critical periods of development, during adulthood, or if the disease represents the cumulative effects of damage throughout the lifespan. To investigate a neurodevelopmental etiology in an animal model of PD, whereby prenatal exposure to previously identified pesticides were hypothesized to disrupt the development of the nigrostriatal DAergic system and enhance its vulnerability to neurotoxicant exposures later-in-life, pregnant C57BL/6J mice were exposed to either saline, or the pesticides maneb (MB, 1 mg/kg), or paraquat (PQ, 0.3 mg/kg). When offspring were evaluated in adulthood, there were no significant effects of prenatal MB or PQ exposure on locomotor activity. Subsequently, offspring were treated for 8 consecutive days with saline, MB (30 mg/kg), or PQ (5 mg/kg). One week after the last exposure, only males exposed to prenatal MB and adulthood PQ showed significant reductions in locomotor activity (95%), changes in striatal neurochemistry, and selective DAergic-neuron loss in the substantia nigra pars compacta. These results suggest that prenatal exposure to MB produces selective, permanent alterations of the nigrostriatal DAergic system and enhances adult susceptibility to PQ exposure, in a gender-dependent way. This study implicates a role for developmental neurotoxicant exposure in the induction of neurodegenerative disorders such as PD, and future work will characterize the state of “silent neurotoxicity” to determine the extent to which it may serve as a pre-symptomatic model framework to further understand the etiology of PD.
Transplantation of Marrow Stromal Cells to the Adult Brain: Long-Term Survival and Region-Specific Gene Expression.

Coyne, T. M. (1), (2); Marcus, A. J. (2); Kramer, B. (2); Woodbury, D. (2); Black, I. B. (2)

(1) Joint Graduate Program in Toxicology, Rutgers University/UMDNJ

(2) Department of Neuroscience and Cell Biology and the Stem Cell Research Center, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ

Adult bone marrow stromal cells (MSC) exhibit stem cell properties, differentiating into multiple mesenchymal derivatives. Emerging evidence in vitro has revealed the differentiation of MSCs into neurons, illustrating far-greater plasticity than previously recognized. We recently extended this evidence in vivo after transplantation of MSCs to the neurogenetic embryonic brain. To determine plasticity in the adult brain, we transplanted 50,000 BrdU-labeled MSCs into the intact adult hippocampus or striatum, which subserve memory and motor function, respectively. We evaluated long-term survival and neuroglial gene expression post-operatively. Survival was remarkably robust at 2 and 12 weeks within the 9-12 mm³ graft site with approximately 75,000 and 50,000 donor MSCs present, respectively. To define the phenotype of grafted MSCs, we evaluated general versus region-specific gene expression at 12 weeks. Overall, 9.4% of donor MSCs in the hippocampus, and 6.5% in the striatum, expressed the mature neuronal marker NeuN. In both regions, nearly 18% of grafted MSCs expressed the glial marker GFAP. Moreover, grafted MSCs exhibited region-specific patterns of differentiation. For example, in the hippocampus, 34.7% of MSCs integrated within the dentate granule layers expressed NeuN, while only 6% expressed GFAP. Conversely, in the surrounding hippocampal regions, a five to one ratio of GFAP-positive to NeuN-positive donor cells was observed. In the striatum, neuroglial gene expression was related to distance from the needle tract. A greater percentage of NeuN-positive MSCs, 7.8%, were observed at distances > 0.5 mm from the needle tract, while a greater percentage of GFAP-positive cells, 34.1%, were observed closer to the tract. Furthermore, expression of genes specific to mature neuronal subtypes was observed in both regions: multiple calbindin-positive MSCs were found in the dentate gyrus, and striatum. Additionally, expression of GAD was observed in donor cells throughout the striatum. These results suggest adult MSCs display remarkable plasticity in vivo, exhibiting long-term survival, integration, and region-specific differentiation after transplantation to the adult brain.
Topoisomerase I-mediated DNA Damage Induced by Oxidative Stress.

Daroui, P.; Liu, L.

Department of Pharmacology, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Piscataway, NJ

Reactive oxygen species modify DNA, generating various DNA lesions including modified bases such as 8-oxoguanine (8-oxoG). These base-modified DNA lesions have been shown to trap DNA topoisomerase I (TOP1) into covalent cleavage complexes. In this study, we have investigated the role of TOP1 in hydrogen peroxide toxicity. We showed that ectopic expression of TOP1 in Saccharomyces cerevisiae conferred sensitivity to hydrogen peroxide, and this sensitivity was dependent on RAD9 checkpoint function. Moreover, in the mammalian cell culture system, hydrogen peroxide-induced growth inhibition and apoptosis were shown to be partly TOP1-dependent as evidenced by a specific increase in resistance to hydrogen peroxide in TOP1-deficient P388/CPT45 murine leukemia cells as compared with their TOP1-proficient parental cell line P388. In addition, hydrogen peroxide was shown to induce TOP1-DNA cross-links. These results support a model in which hydrogen peroxide promotes the trapping of TOP1 on oxidative DNA lesions to form TOP1-DNA cleavage complexes that contribute to hydrogen peroxide toxicity.
Immune Regulatory Mechanisms in Host-Tumor Interaction.

Gabriel, E. M.; Monken, C. E.; Lattime, E. C.

The Cancer Institute of New Jersey, UMDNJ-Robert Wood Johnson Medical School, Department of Surgical Oncology, UMDNJ Graduate School of Biomedical Sciences, Department of Molecular Genetics, Microbiology, & Immunology, Piscataway, NJ

We have investigated the roles of two immune regulatory mechanisms, CD4+CD25+ regulatory T cells (Treg cells) and the cytokine IL-10, in the host-tumor interaction to optimize our vaccinia virus-based anti-tumor vaccine strategies. Studies have shown that depletion of the Treg cell population results in tolerance to tumor formation. We have previously shown that IL10 KO mice also tolerate tumor formation. In this most recent project, we treated 6-8 naive female C57BL/6J (B6) mice with either anti-CD25 monoclonal antibody or anti-IL10 monoclonal antibody followed by subcutaneous injection of the mouse bladder cancer MB49, which naturally expresses the male antigen HY. We monitored tumor formation, and performed cytotoxic T-cell chromium-51 release assays to measure functional anti-tumor activity against MB49 and flow cytometry analysis using PE-conjugated tetramer specific for HY-recognizing TCR. Female B6 treated with anti-CD25 antibody had smaller tumor areas than controls at the end-point (55.56 mm2 versus 255.17 mm2), whereas female B6 treated with anti-IL10 antibody showed similar tumor areas to controls (256.03 mm2 versus 254.58 mm2). T-cells from female B6 treated with anti-CD25 antibody showed greater anti-tumor activity compared to controls (77.38 percent specific lysis versus less than 5 percent) and had a greater percentage of tetramer positive cells compared to controls (6.71 percent versus 1.50 percent). In contrast, T-cells from female B6 treated with anti-IL10 antibody showed similar anti-tumor activity compared to controls (7.17 percent specific lysis versus 9.16 percent). We conclude that depletion of the Treg cell population modulates the immune response against tumor by decreasing the regulatory actions of Treg cells. The depletion of IL10, however, was not sufficient to induce tumor rejection. We will further expand the findings of this project by combining the anti-CD25 antibody therapy with our established vaccinia virus-based vaccines.
Image-Guided Decision Support in Diagnostic Pathology.

Hall, B. H.; Foran, D. J.

Center for Biomedical Imaging & Informatics

UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Piscataway, NJ

A diagnosis impacts how aggressively patients are treated, which therapies are appropriate, and what levels of risk are justified. In some areas of hematopathology, clinical decisions can only be rendered after performing immunophenotyping and/or molecular studies. Unfortunately, such supporting analysis is generally performed only after microscopic evaluation causes one to first classify the specimen as suspicious. While it is currently impractical to perform ancillary testing on every specimen, a reliable, image-based screening system could potentially reduce costs, patient morbidity and mortality. During feasibility studies, a computer-based prototype was developed to automatically generate the spectral and spatial signature of the underlying pathology of imaged cells based on Fourier descriptors, multivariate color metrics, and multi-resolution texture attributes. Using multivariate data fusion a weighted mixture of these features served as search criteria to retrieve the diagnoses, correlated clinical data, and image records of those cases from within a distributed database of “gold standard” cases which exhibited profiles which were consistent with those of the undiagnosed case. The prototype provided the correct classification in 83% of trials using a mixed set of malignant lymphomas and leukemias which can sometimes be confused with one another during routine microscopic evaluation (Chronic Lymphocytic Leukemia, Mantle Cell Lymphoma, Follicular Center Cell Lymphoma and benign). In an attempt to refine the discriminatory power of the system, we are investigating the use of texton histogram signatures to distinguish the cellular characteristics of the original test set of lymphoproliferative disorders and a set of acute leukemias.

A texton is defined as conspicuous repetitive local feature in human texture perception. The computational model for textons are clusters which are generated in multivariate feature space in response to a fixed set of filter banks. We are currently conducting a systematic investigation to determine the preferred filter banks and optimal number of modes for differentiating among a spectrum of routine and rare malignancies. Further, we plan to utilize statistical pattern recognition to determine if there exists a relationship between the morphologic and clinical (protein /molecular) profiles of these disorders and to explore the use of textons in characterizing expression patterns in cancer tissue microarrays.
A Tissue Engineered Meniscus Replacement.

Langhammer, C., Gatt Jr., C. J.

Department of Orthopaedic Surgery, UMDNJ-Robert Wood Johnson, Medical School, Picataway, NJ

The menisci play many important roles in the healthy functioning of the knee, including load bearing, force distribution, joint stabilization, joint lubrication, and proprioception. Damage to meniscal cartilage is associated with detrimental changes in joint function that can lead to pain, disability, and degenerative osteoarthritis. The function of meniscal cartilage as a weight-bearing structure depends on the specific arrangement of collagen fibers. Collagen bundles are arranged circumferentially in the core of the meniscus and in disordered or lamellar bundles on and just below the surface, explaining the high frequency of circular tears seen in practice. Despite the recognized importance of the meniscus, no current treatment has been shown to be successful in the long term rehabilitation of patients with meniscal defects. Subtotal meniscectomy remains one of the most commonly performed orthopaedic procedures regardless of its poor long-term outcomes. Our laboratory is researching techniques for the design and fabrication of a tissue engineered meniscus. The object of tissue engineering is to use an artificial scaffold material to guide the growth of host tissue in such a way to promote wound healing and replacement of the graft by viable host tissue. Because of the mechanical stresses placed on the meniscus, special consideration must be used when assessing materials to be used in such an application. We are using a bio-resorbable synthetic polymer fiber to reinforce synthetically cross-linked collagen sponges, altering their mechanical properties. We are varying the density and pattern of fiber distribution and using standard mechanical testing techniques to examine the effect on sponge mechanical properties.
Regional and Gender Specific Effects of Estradiol on Astrocytes.

Livingston, D. L.; Lackland, J. C.; Dreyfus, C. F.

Department of Neuroscience, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Piscataway, NJ.

Our studies have shown that estradiol increases BDNF expression in astrocytes. These effects are regionally heterogeneous, with post-natal day one (P1) rat hippocampal and caudal cortical astrocytes increasing BDNF expression after estradiol treatment, while rostral cortical and basal forebrain astrocytes do not respond. Moreover, we have found that this effect is gender specific. Female but not male P1 hippocampal astrocytes increase BDNF expression after estradiol treatment. To define receptors mediating these effects, the presence of estrogen receptor alpha and beta were investigated using western blot, immunocytochemistry and RT-PCR. These astrocytes express both receptors and are therefore in position to respond directly to estradiol when treated for 24 hours. Estrogen receptor beta agonists, DPN and genistein elicit increased BDNF expression. On the other hand, estrogen receptor alpha agonists 16-alpha-iodo estradiol (gift of R. Hochberg) and PPT do not. These preliminary agonist studies suggest that estrogen receptor beta mediates increases in BDNF expression in neonatal hippocampal astrocytes. Since BDNF is known to affect synaptic plasticity in the hippocampus, these results suggest that there are gender related differences in the development of hippocampal circuitry, which are mediated through estrogen receptor beta.

Supported by NIH HD23315
Adult Bone Marrow Stromal Cells in the Embryonic Brain: Long-Term Survival and Sub-region Specific Differentiation.

Marcus, A. J.; Coyne, T.; Woodbury, D. (1); Black, I. B. (1)

Department Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Stem Cell Institute of New Jersey, Piscataway, NJ

We have recently characterized postnatal engraftment, migration and phenotypic expression after infusion of undifferentiated bone marrow stromal cells (MSCs) into E15.5 (embryonic day) rat telencephalic ventricles in utero. BrdU (bromodeoxyuridine)-labeled MSCs were observed at one and two months postnatally in a number of brain areas including the upper laminae of the neocortex, periventricular area (ependyma, subependymal layer, and subventricular zone), and olfactory bulb. At these times, an abundance of donor MSCs expressed NeuN, $\alpha$-III-tubulin, NF-M (intermediate weight neural filament protein), and nestin. To obtain a quantitative estimate of survival and differentiation in the postnatal animal, confocal microscopy was performed. Thousands of donor cells per cubic mm survived and engrafted at one month. Analysis of the neocortex and olfactory bulb revealed that 98.6 and 77.3% of the donor MSCs were NeuN (neuronal-specific nuclear protein) positive. Here we have examined the olfactory bulb to determine whether MSCs exhibit subregion-specific differentiation and gene expression. Appropriately, calbindin-positive BrdU-labeled MSCs were observed in the external plexiform layer, and tyrosine hydroxylase-positive donor cells were observed in a separate subregion of the periglomerular layer. We have also examined survival of transplanted MSCs in older animals. MSCs survived at least one year postnatally, the longest time examined. This indicates long-term persistence of the transplanted MSCs in the adult brain. Our studies demonstrate that MSCs have the capacity to migrate, engraft, and differentiate into region-specific neurons following transplantation into the embryonic rat brain. Long-term survival of MSCs may afford the opportunity for new in utero therapies for both congenital and degenerative neurological diseases.
Expression of Pokeweed Antiviral Protein: Insights into the Endoplasmic Reticulum Associated Degradation Pathway.

Parikh, B. A.; Tumer, N. E.

Department of Molecular Genetics, Microbiology, & Immunology, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Piscataway, NJ and Rutgers University, Graduate School New Brunswick, New Brunswick, NJ

Pokeweed antiviral protein (PAP) is a single-chain ribosome inactivating protein (RIP) that binds to ribosomes and depurinates the highly conserved •-sarcin/ricin loop of the large subunit rRNA. Catalytic depurination of a specific adenine residue has been proposed to result in translation arrest and cytotoxicity. Many plant and bacterial toxins including ricin, shiga toxin, pertussis toxin, pseudomonas exotoxin A, and cholera toxin have been postulated to gain entry into the cytoplasm and exert their cytotoxic effects by evading the host cell protein degradation machinery. Elaborate evasion strategies involving both the endoplasmic reticulum (ER) and the proteasome have been shown to play crucial roles.

This is the first report that PAP also utilizes a similar form of evasion strategy to promote cytosolic transport, avoid degradation, and account for its unusual stability. We demonstrated that the precursor form of PAP accumulates on ER membranes in yeast and the mature form is retrotranslocated from the ER into the cytosol where it escapes degradation. We also hypothesize that the mechanism of proteasome evasion is different from that already described for ricin. Recent advances in the ER-associated degradation (ERAD) pathway will be reviewed. Additionally the medical significance of the ERAD pathway as its potential for the modulation of human diseases will be discussed.
Na+/H+ Exchanger Inhibition Protects Cultured Rat Mesencephalic Neurons Against Malonate Toxicity.

Rocha, M.; Bernard, L. P.; Zeevalk, G. D.; Sonsalla, P. K.

Department of Neurology, UMDNJ-Robert Wood Johnson Medical School-Graduate School of Biomedical Sciences, Piscataway, NJ

Mitochondrial defects are associated with the pathogenesis of Parkinson’s disease and previous studies have implicated the plasma membrane Na+/H+ exchanger (NHE) in brain tissue damage following energy failure. This has raised the hypothesis that modulation of NHE activity may protect dopaminergic neurons against metabolic stress. To test this hypothesis, we have examined the viability of cultured rat mesencephalic neurons challenged with malonate, a reversible mitochondrial inhibitor, during NHE inhibition with 5-(N-ethyl-N-isopropyl)amiloride (EIPA). Cultures that were exposed to malonate exhibited a significant loss of high affinity uptake for 3H-DA and 14C-GABA in a dose-related manner. EIPA significantly attenuated the malonate-induced loss of 3H-DA and 14C-GABA uptake at concentrations that inhibit NHE activity in vitro. In other experiments, we began investigating the mechanisms by which NHE inhibition may protect neurons against malonate toxicity. The intracellular pH (pHi) of rat mesencephalic cell populations was monitored in HEPES-buffered solution using the H+-sensitive probe 2’,7’-bis-(2-carboxyethyl)-5-(and-6)-carboxyfluorescein. Cultures exhibited a rapid cytosolic alkalinization relative to baseline pHi when exposed to malonate. While EIPA alone did not appear to affect baseline pHi, it attenuated the malonate-related cytosolic alkalinization. Additional studies are required to determine exactly how NHE inhibition protects against the loss of neuronal viability due to energy impairment. However, these findings suggest that NHE activity contributes to the toxicity imposed by a metabolic stress to cultured mesencephalic neurons. Most importantly, the NHE may represent a potential target for protecting dopaminergic neurons against mitochondrial dysfunction, and this idea is currently being tested in vivo.

This work was supported by NIH grants AG08479, NS41545, R25NS25952-15 (Neuroscience Scholars Program) and a RWJMS summer fellowship award.
En2, an Autism-Associated Gene, Regulates Cerebellar Granule Neuron Precursor Responses to Extracellular Signaling Molecules During Development.

Rossman, I.; Kamdar, S.; Millonig, J.; DiCicco-Bloom, E.

Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, and Graduate School of Biomedical Sciences, Piscataway, NJ

Autism Spectrum Disorder (ASD) is a complex developmental disease, characterized by behavioral, intellectual, and language deficits, that spans all races and socioeconomic levels with prevalence greater than 1 in 166 births. While the symptomology of ASD varies, postmortem studies have identified the cerebellum as the most consistent site of ASD neuropathology. The underlying mechanisms responsible for ASD pathogenesis remain unknown, however both environmental and genetic etiologies have been proposed.

Engrailed 2 (En2) is a mammalian patterning gene whose complex spatial and temporal expression is required for normal cerebellar development. Genetic disruption of En2 yields cerebellar hypoplasia with deficient Purkinje and granule cells, similar to the neuropathology found in ASD. Recently, Gharani et al. (2004) found autistic individuals inherited specific polymorphisms in human EN2 much more frequently (p<0.0001) than non-autistic siblings, a finding replicated in two additional datasets. These data suggest EN2 is an autism-susceptibility locus, thus understanding the developmental role of EN2 may facilitate our understanding and treatment of ASD.

To define En2 functions, we examined DNA synthesis in whole cerebellum and granule neuron precursors (GNPs) in vivo, as well as GNPs in culture in response to growth signals, using C57/BL6 (WT) and En2 knockout (KO) mouse pups. GNPs were isolated by genotype, cultured for 24h in defined media, and DNA synthesis was assessed by 3H-deoxythymidine (3H-dT) or BrdU incorporation. Proliferation in vivo was reduced in whole KO cerebellum and GNPs, suggesting disordered growth regulation. In culture, no genotype-dependent differences in baseline GNP mitosis were observed. However, IGF1 elicited a 2-fold increase in DNA synthesis in WT, and a 6-fold increase in KO, with parallel results obtained with BrdU labeling, suggesting normal En2 expression negatively regulates IGF1 mitogenic signaling. Consistent with this model, KO GNPs exhibited decreased process outgrowth (WT=42%; KO=25%; % neurite-bearing cells). There were no genotype-dependent differences in cell survival. No other growth factors engaging tyrosine kinase receptors (bFGF, BDNF, EGF) nor non-kinase receptors (Shh, PACAP) elicited genotype-dependent effects, suggesting that overall cell cycle regulation is comparable among genotypes. Together, these data identify previously unrecognized interactions between IGF1 and En2 expression, suggesting altered En2 activity may contribute to abnormal cerebellar growth via aberrant IGF1 signaling.

Supported by: NINDS F30 NS048649-01; ES11246; USEPA-R829391; NJ Gov Coun. Autism.
Tropomyosin has a Co-Requirement of Coiled Coil Flexibility and Specific Non-Interface Residues for Actin Binding.

Singh, A.; Hitchcock-DeGregori, S.

Department of Neuroscience and Cell Biology, UMDNJ-Robert Wood Johnson Medical School, Piscataway, NJ

Tropomyosin (TM) is a coiled coil along its length, with subtle variations in the structure to allow interactions with actin and other binding proteins. Analysis of TM's sequence reveals a seven-fold repeat (Phillips, 1986; McLachlan and Stewart -zones) that corresponds to actin binding periods that are not functionally quasiequivalent. We are investigating how specificity is imparted to the coiled coil structure. In tropomyosin, the middle periods are important for cooperative activation of actin by myosin, in particular a highly-conserved region, residues 166-186, that contains two cardiomyopathy-causing mutations. This region, period 5, includes an Ala cluster, a motif rich in interface alanines, and along with period 1, is the only one with the cluster within an -zone. In previous work we replaced residues 166-186 with a GCN4 leucine zipper that destroyed function. Introduction of AAS or QNQ residues at the leucine zipper interface partially restored actin binding and cooperative myosin-induced binding. Additional replacement of non-interface residues proposed to be important for actin binding (Phillips, 1986) further restored function, showing that flexibility is necessary but not sufficient for function. Replacement of part of period 1 into period 5 resulted in almost normal function with an increase in global stability. However, replacement of period 5 with part of period 2, in which the sequence was slightly out of register, resulted in loss of function with wildtype global stability. These mutants further underscore the functional specificity of the -zone of period 5, and also the importance of the similarity between period 1 and 5. Periods 1 and 5 are the least typical of the seven, but contain the most conserved regions in tropomyosin. These results suggest that to bind to actin there must be local instability (flexibility) in the coiled coil within an -zone to allow for the non-interface side chains to bind to actin.

Supported by NIGMS.
Integration of Mitochondrial on DNA in Yeast.

Telesford, N.; Gabriel, A.

Department of Biochemistry, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, and Rutgers University, Piscataway, NJ

Our laboratory studies the mechanisms of double strand break (DSB) repair in the yeast Saccharomyces cerevisiae. In our URA3 –actin intron assay system we create chromosomal DSBs using either HO or I-Sce1 endonuclease. We are particularly interested in extrachromosomal DNA that can be inserted at DSBs during their repair. Previous work in the lab showed that in the presence of the HO endonuclease, 90% of the inserts were Ty1 elements. Ty1 elements are retrotransposons that are a ubiquitous family of transposable elements that are transcribed to mRNA. This mRNA is then copied into cDNA through element-encoded reverse transcriptase (RT) and the cDNA is able to integrate into new locations in the host genome. The profile of the other 10% of the inserts, were of mitochondrial origin. In the presence of a DSB generated by I-Sce1, the opposite occurred, with 90% of inserts being of mitochondrial origin and 10% Ty1. Further it was found that many of these mitochondrial fragments clustered around transcription initiation sites and mobile introns in the mitochondrial genome. Mobile introns fall into two categories – Group I and Group II mobile introns. Group I introns are autonomous self-splicing elements that catalyze (i.e. they are ribozymes) their own removal from RNA transcripts by forming complex secondary and tertiary structures. Group II introns are novel genetic elements that have properties of both catalytic RNAs and retroelements. Our result points to the possible involvement of RT in the creation of mitochondria cDNA that then integrate at DSB. I will be studying the mechanism of mt DNA integration during my doctoral studies. In particular, I will examine the role of RT associated with Group II introns in generating mitochondrial fragments. Second, I will explore the role of the mitochondria and how it relates to DSB repair under anaerobic conditions. Third, I will examine the effect of mitochondrial apoptosis in DSB repair. These studies on mitochondrial integration at DSBs will provide new insight into mechanism of mtDNA movement into the nuclear genome.
Racial Disparities in Hepatitis C. Testing.

Trooskin, S.

UMDNJ-Robert Wood Johnson Medical School, UMDNJ-School of Public Health, Piscataway, NJ

Background: Previous studies showed that physicians do not adequately screen and test for hepatitis C (Sehab, 2003). To explore the possibility that additional barriers exist to HCV testing, we examined whether race, trust in physicians, health beliefs, and knowledge of hepatitis C are associated with rates of testing for HCV, in the presence of an identified risk factor for HCV acquisition.

Methods: We prospectively enrolled all patients, 18 or older, who had a risk factor for acquisition of HCV, and were being seen for the first time at any of four primary care practices within the greater Philadelphia area. These practices included two federally qualified health clinics serving predominantly minority populations and two university-based primary care practices, one of which was a family medicine practice and the other was an internal medicine practice. Questionnaires assessing demographic information, trust in physicians, patients' fatalistic beliefs about their health, and knowledge of HCV were completed by participants. Chart reviews were conducted 2 months after the physician visit to assess if HCV testing was planned or performed.

Results: Of the 1415 patients enrolled in this study, 512 participants identified themselves as Caucasian, 468 as Latino or Hispanic origin, and 435 as African American. Risk factor status differed by race, with 39% of Caucasians, 31% of Hispanics, and 48% of African Americans reporting a risk factor. Eight percent of African American patients with a risk factor were tested for HCV as compared to 11% of Caucasians and 20% of Hispanics. After adjusting for insurance status and education in the multiple covariate model, both increasing age and race remain significant predictors of HCV testing. Hispanics born in the United States (including Puerto Rico) were no more likely to be tested in the presence of a risk factor than non-US born Hispanics. We found that trust in physicians, patient’s health beliefs, and HCV knowledge were not significant predictors of testing.

Discussion: Race alone is a major barrier to HCV testing, even in the presence of a risk for acquisition; patients’ trust in physicians, health beliefs, and HCV knowledge do not affect their willingness to be tested. Further investigation is required to explain low testing rates among African Americans at risk for HCV.

Williams T. A.; Johnson, W.

Department of Neurology, UMDNJ-Robert Wood Johnson Medical School and Graduate School of Biomedical Sciences, Piscataway, NJ

Autistic disorder (AUT) is a pervasive developmental disorder associated with abnormalities of higher neurologic functions, e.g. language delay, aberrant social interactions, and repetitive behaviors. AUT pathogenesis has been linked to increased immune activation, abnormal antioxidant mechanisms, and excessive brain growth during development. AUT is suggested to have multiple genetic risk factors. The overall hypothesis of this study was that certain genetic factors affecting immune status, brain growth, and antioxidant mechanisms could be risk factors for AUT. These three pathways are inter-related and feedback upon one another. Therefore, genetic changes in one may also affect the others.

The hypothesis was first tested by investigation of potential elevation of immune function in individuals with AUT and their parents as compared with controls. There was found to be a significant increase in the number of individuals with detectable plasma levels of tumor necrosis factor (TNF) in affected individuals and fathers compared with controls.

The hypothesis was then tested by investigation of genetic polymorphisms in case-parent trios. One antioxidant gene, glutathione-S-transferase mu 1 (GSTM1) has a null allele polymorphism that is complete deletion of the gene. The homozygous null genotype occurred significantly more commonly in AUT probands and fathers vs. controls and may be a modifying/specificity allele.

The hypothesis was finally tested by investigation of polymorphisms in the mothers and maternal grandparents of affected individuals for increased transmission of genetic factors that may be acting in the mothers during pregnancy to harm the fetus. For the non-deletion allele of a polymorphic 19-bp deletion in intron 1 of dihydrofolate reductase (DHFR), the homozygous genotype for this allele was present significantly more often in mothers compared with controls. We also found significance for the transmission of a two-allele haplotype of a GST pi gene, GSTP1*A, in AUT maternal trios. These findings are suggestive for a role of the DHFR non-deletion allele as well as the GSTP1*A haplotype as teratogenic alleles.

In summary, genetic risk factors of the innate immune pathway and the related folate and antioxidant pathways may contribute to AUT etiology, either by direct genetic effects in the proband or by teratogenic effects during gestation.
MD/PhD Students

Fall 2005
<table>
<thead>
<tr>
<th>Name</th>
<th>Year</th>
<th>Program</th>
<th>Email</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abbound, Jean-Paul</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Abboudjj@umdnj.edu">Abboudjj@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Anton, Kevin</td>
<td>2nd year RWJMS</td>
<td><a href="mailto:Antonkf@umdnj.edu">Antonkf@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Barlow, Brian K.</td>
<td>2nd year RWJMS</td>
<td><a href="mailto:Barlowbr@umdnj.edu">Barlowbr@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Coyne, Thomas M.</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Coynetm@umdnj.ed">Coynetm@umdnj.ed</a></td>
<td></td>
</tr>
<tr>
<td>Darouei, Parima</td>
<td>4th year RWJMS</td>
<td><a href="mailto:Darouipa@umdnj.edu">Darouipa@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Gabriel, Emmanuel M.</td>
<td>2nd year GSBS/RWJMS</td>
<td><a href="mailto:Gabrieem@umdnj.edu">Gabrieem@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Haley, Erin</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Ehaley@loyola.edu">Ehaley@loyola.edu</a></td>
<td></td>
</tr>
<tr>
<td>Hall, Bonnie</td>
<td>2nd year GSBS/RWJMS</td>
<td><a href="mailto:Huangbo@umdnj.edu">Huangbo@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Langhammer, Christopher</td>
<td>2nd year RWJMS</td>
<td><a href="mailto:Langhach@umdnj.edu">Langhach@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Liu, Sean</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Sthliu@yahoo.com">Sthliu@yahoo.com</a></td>
<td></td>
</tr>
<tr>
<td>Livingston, Denise L.</td>
<td>4th year GSBS/RWJMS</td>
<td><a href="mailto:Livingdl@umdnj.edu">Livingdl@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Marcus, Akiva J.</td>
<td>3rd year GSBS/RWJMS</td>
<td><a href="mailto:Marcusak@umdnj.edu">Marcusak@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Mazari, Peter</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Petemaz@hotmail.com">Petemaz@hotmail.com</a></td>
<td></td>
</tr>
<tr>
<td>Parikh, Bijal A.</td>
<td>4th year RWJMS</td>
<td><a href="mailto:Parikhbhi@umdnj.edu">Parikhbhi@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Rocha, Marcelo</td>
<td>2nd year GSBS/RWJMS</td>
<td><a href="mailto:Rochama@umdnj.edu">Rochama@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Rossman, Ian</td>
<td>3rd year GSBS/RWJMS</td>
<td><a href="mailto:Rossmaia@umdnj.edu">Rossmaia@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Sethi, Nilay</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Nsethi@princeton.edu">Nsethi@princeton.edu</a></td>
<td></td>
</tr>
<tr>
<td>Singh, Abhishek</td>
<td>1st year GSBS/RWJMS</td>
<td><a href="mailto:Singha2@umdnj.edu">Singha2@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Suh, Jean</td>
<td>1st year RWJMS</td>
<td><a href="mailto:Hs86@cornell.edu">Hs86@cornell.edu</a></td>
<td></td>
</tr>
<tr>
<td>Telesford, Natasha</td>
<td>4th year GSBS/RU</td>
<td><a href="mailto:Telesfna@umdnj.edu">Telesfna@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Treiser, Matthew</td>
<td>1st year RU</td>
<td><a href="mailto:Treiseml@umdnj.edu">Treiseml@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Troosskin, Stacey</td>
<td>3rd year RWJMS</td>
<td><a href="mailto:Trooskst@umdnj.edu">Trooskst@umdnj.edu</a></td>
<td></td>
</tr>
<tr>
<td>Williams, Tanishia A.</td>
<td>4th year RWJMS</td>
<td><a href="mailto:Williata@umdnj.edu">Williata@umdnj.edu</a></td>
<td></td>
</tr>
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</table>
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