

Charles H. Hood Foundation

July 2010 Award Recipients

- **Abraham Brass, M.D., Ph.D.**
Instructor in Medicine
Massachusetts General Hospital

“Understanding Intrinsic Immunity: Investigation of IFITM3's Inhibition of Influenza A Virus Infection”

Key Words: influenza A Virus, Pediatric Influenza, Viral Host Interactions, Intrinsic Immunity, Interferon, Restriction Factor

Influenza epidemics exact a formidable toll on world health and disproportionately effect the very young and old. At present, the emergence of a novel influenza A H1N1 viral strain has created a pandemic, producing illness in over 200 countries. To find host-cell modifiers of influenza A H1N1 viral infection, we completed a large scale genetic screen and detected several proteins which are important in decreasing influenza A virus infection, including a role for Interferon-inducible trans-membrane protein 3 (IFITM3). The loss of IFITM3 resulted in elevated viral replication in multiple cell lines tested, and proved to be critical for IFN-induced viral resistance, accounting for 40% to 70% of IFN's protective ability. IFITM3 belongs to a family of four closely related proteins in humans, and five proteins in mice. This application aims to elucidate the role of the IFITM proteins in the host response to viral infection. Successfully achieving the aims of this proposal will provide an in depth knowledge of the actions of IFITM3 and will inform us more fully about our intrinsic immune response to viruses. In our first aim we seek to define the mechanism of IFITM3-mediated inhibition of viral infection using functional, structural and image-based studies. The experiments in aim 1 will improve our understanding of how the IFITM3 stops viruses, and therefore may suggest new ways to prevent or treat influenza. Our second aim is designed to detect protein interactions involving IFITM3 and rigorously test the functional relevance of these connections. We expect these studies to identify new effectors of cell intrinsic immunity and also deepen our understanding of the actions of IFITM3. Fulfilling these two aims will further the project's long term goal of understanding how our cells defend themselves against viral invasion and may provide new tools and strategies for stopping infections.

- **Thomas Murray, M.D., Ph.D.**
Associate Research Scientist of Pediatrics and Laboratory Medicine
Yale University

“The Role of Lactate Metabolism in Pseudomonas Aeruginosa Biofilm Formation”

Key Words: Pseudomonas Aeruginosa, Lactate, Biofilm, Cystic Fibrosis

Chronic pulmonary infection with Pseudomonas aeruginosa causes recurrent hospitalization and mortality in children with cystic fibrosis (CF). These chronic infections are difficult to eradicate because P.aeruginosa forms organized

biofilms encased in a protective, extracellular matrix composed of exopolysaccharide. Altering the nutritional environment can change the biofilm structure, increasing the susceptibility of *P.aeruginosa* biofilms to antibiotics. Our long term goal is to identify novel therapeutic targets to treat chronic infection in children with CF, either by manipulating an environmental factor required for biofilm formation or by disrupting bacterial pathways that trigger biofilm formation. Evidence from other bacteria shows extracellular lactate, a potential energy source for *P.aeruginosa* elevated in the bronchoalveolar lavage fluid from CF patients, is an important determinant of biofilm formation and in vivo colonization. We have identified a novel mutation in a predicted *P.aeruginosa* lactate permease (IldP), which alters biofilm formation. We hypothesize that lactate metabolism is one factor that determines *P.aeruginosa* biofilm formation and represents a potential therapeutic target. The specific aims of this study are to: 1) Determine the mechanism by which lactate metabolism influences *P.aeruginosa* biofilm formation. We will measure lactate uptake and metabolism, examine biofilm formation, and measure exopolysaccharide synthesis in wild type *P.aeruginosa* and in mutant strains lacking either the lactate permease or related lactate oxidase. These experiments will be conducted with varied extracellular lactate levels to understand how changing extracellular lactate alters biofilm formation. 2) Determine whether the in vitro defects in biofilm formation due to altered lactate uptake are important for mammalian lung infection. Using a novel CF murine model of lipopolysaccharide induced chronic inflammation, wild type and CF mice will be infected with wild type *P.aeruginosa* or a strain lacking the lactate permease and the lungs examined for the presence of bacteria and inflammation.

- **Lise Nigrovic, M.D., M.P.H.**

Assistant Professor
Children's Hospital Boston

"Development and Pilot Testing of a Computerized Decision Rule for Children with Minor Blunt Head Trauma"

Key Words: Clinician Decision Rule Implementation, Automated Decision Support, Blunt Head Trauma, Radiation Risk

We propose to develop and pilot test a computerized decision support tool for the care of children with blunt head trauma in the emergency department (ED) environment. We will measure our ability to deliver the decision support tool for the care of children with minor blunt head trauma at the point of decision making and order entry for cranial computed tomography (CT).

While minor head trauma is a common reason for ED evaluation of children, the prevalence of clinically important traumatic brain injury requiring intervention is very low. Utilization of cranial CT for evaluation of children after minor head trauma has been steadily increasing over the past decade. An emergent CT, however, is not without substantial risks. The most important risk is the long-term induction of lethal malignancy resulting from the radiation exposure associated with CT scans.

The Pediatric Emergency Care Applied Research Network recently published a validated clinical decision rule for the care of children with blunt head trauma

utilizing a prospective cohort of almost 45,000 children with blunt head trauma evaluated in the ED. The rule identifies children at very low risk of clinically important traumatic brain injury who may not need acute neuro-imaging. We propose to develop and then pilot test a computerized decision support tool for the published PECARN head trauma rule. We will deliver this decision support at the time of cranial CT decision making. In addition, we expect that the knowledge gained from this study will inform a subsequent larger multi-center implementation study.

- **In-Hyun Park, Ph.D.**

Assistant Professor
Yale University

“Investigation of Functional Myogenic Progenitors from Human ES and iPS Cells for Duchenne Muscular Dystrophy”

Key Words: Reprogramming, iPS Cells, hES Cells, DMD, Myogenic Progenitors

Duchenne muscular dystrophy (DMD) is severe recessive X-linked disorder, and one of the most prevalent pediatric genetic diseases (1 in 3,500 newborn males). Mutations in dystrophin, a major component of the cytoskeletons of muscular fibers, are the underlying cause of DMD, resulting in structural instability within cardiac and skeletal muscle, and accelerates turnover of myogenic stem cell pools. Since the discovery of dystrophin as an underlying gene of DMD, gene and cell therapy were attempted to treat or cure DMD. Lentiviral vectors or adeno- or adeno-associated vectors expressing mini-dystrophin or exon-skipping oligomers showed a limited success in rescuing dystrophic phenotype. Clinical application of physiological myoblasts, satellite cells, showed no adverse but also no effective treatment on DMD patients. Recently preclinical success of mesoangioblastic pericytes in ameliorating muscular dystrophic symptom opens a promising opportunity for systemically transplantable cell-based therapy for DMD. What is critical is to obtain cells histocompatible for patients. Expression of four defined factors (Oct4, Sox2, Klf4, Myc) reprograms somatic cells to become induced pluripotent stem (iPS) cells that potentially allows obtaining autologous myogenic progenitors for DMD patients. Our long-term research goal is to derive patient specific systemically transplantable myogenic progenitors from pluripotent stem cells, as a novel cellular source for DMD patients. From our preliminary investigation, we isolated cells with myogenic potential (MPCs, myogenic progenitor cells) differentiated from human pluripotent stem cells (hPSC). hPSC-MPC showed the heterogeneity of cell populations and we seek to enrich myogenic population that can be transplanted systemically. Following specific aims are proposed; 1) to functionally determine the cell surface phenotype of hPSC-MPCs, and 2) to apply genetic approach to improve the systemic delivery of the hPSC-MPCs into target muscle. Success of our proposed research will provide a robust novel cell source for DMD treatment.

- **Christian Schlieker, Ph.D.**

Assistant Professor of Molecular Biophysics and Biochemistry
Yale University

“Investigating Nuclear Envelopathies from the Perspective of Protein Quality Control”

Key Words: Nuclear Envelopathies, Laminopathies, Protein Misfolding Diseases, Proteotoxicity, Protein Quality Control, Nuclear Envelope, Protein Aggregation, Autophagy

Nuclear envelopathies are a diverse group of congenital diseases that are caused by mutations affecting proteins in the nuclear envelope or lamina. Emery Dreifuss muscular dystrophy and progeria syndromes are not only amongst the most severe forms, they also have a very early onset and therefore affect children severely. Both are caused by mutations in the Lamin A gene. These Lamin A alleles act as dominant negatives and often form protein deposits when overexpressed. However, no phenotype is observed upon genetic ablation of Lamin A in animal models. We therefore hypothesize that envelopathy-associated alleles act at least in part through proteotoxicity, i.e. by a gain of function mechanism that leads to a poisoning of the protein quality control system. How proteins in the nuclear periphery are turned over or repaired is largely unknown, and the mechanisms that serve to remove nuclear protein aggregates are equally elusive.

Our goal is to unravel the cellular mechanisms that regulate protein homeostasis in the nuclear periphery, and to elucidate the role that these pathways play in muscular dystrophies, premature aging and related envelopathies that affect children. To accomplish our goal, we will establish novel model substrates to study protein toxicity and turnover in relation to nuclear envelopathies. Furthermore, we will exploit viral proteins known to manipulate the nuclear envelope as a novel approach to identify cellular factors involved in protein turnover and aggregate removal from the nucleus.

The results obtained from these studies will provide the first molecular insights into the constituents responsible for turnover of protein aggregates in the nucleus. The cellular factors identified in this study will greatly enhance our understanding of nuclear envelopathies and will also serve as a critical step toward the development of therapeutic interventions to improve and possibly extend the life expectancy of children afflicted with these diseases.

January 2010 Award Recipients

- **William Anderson, Ph.D., M.D.**

Instructor of Surgery
Brigham and Women's Hospital

“The Impact of Interictal Spike Events on Visual Object Recognition”

Key Words: Epilepsy, Cognitive Testing, Interictal Spike, Visual Processing

The goal of this proposal is to investigate the link between interictal epileptiform activity and cognitive performance in children. Our aims involve first developing a reliable, automated, intracranial interictal spike detection

algorithm. This will involve decomposing an incoming signal, which in this case is an intracranial electroencephalogram, into its wavelet coefficients, and then instituting an algorithm which detects features of interest. The algorithm will be designed to run online so as to be able to detect interictal spikes in real time on patients undergoing invasive monitoring for resective surgery. The second aim is to investigate whether interictal spikes effect cognitive performance, which in this context will involve tasks related to visual object recognition. The detection of a spike by our automated algorithm, will trigger one of two delay-match-to-sample tasks. These tasks will first involve the presentation of a noisy image, followed by a probe image taken from a pre-assigned database of images. Our results will be controlled against two other experimental conditions which present images not ostensibly time-locked to interictal spike detection. If successful, this proposal will represent a significant contribution to the body of evidence supporting the detrimental effects of interictal activity on cognition.

- **David Guertin, Ph.D.**

Assistant Professor

University of Massachusetts Medical School

“Nutrient Sensing Pathways in Muscle Regeneration”

Key Words: mTOR, mTORC1, mTORC2, Raptor, Rictor, Rapamycin, PI3K, PTEN, Muscle Regeneration, Satellite Cells, Stem Cells, SMPs, Skeletal Muscle Precursors, Muscular Dystrophy, Rhabdomyosarcoma

The broad, long-term goal of this project is to identify signaling pathways that can be manipulated to grow stem cells efficiently in culture. Our current focus is on the nutrient and growth factor sensing mTOR pathway and its role in regulating skeletal muscle stem cell function. In preliminary studies, we find that mTOR may regulate the self-renewal and differentiation of skeletal muscle precursor cells (SMPs), which are thought to represent the skeletal muscle stem cell pool. SMPs prospectively isolated from adult skeletal muscle can be transplanted and engrafted into recipient mice, thus providing a potential source of transplantable stem cells for treating muscle degenerative diseases. Fully understanding the mechanisms controlling SMP proliferation, self-renewal and differentiation is critical to making this reality.

Our objective in this proposal is to comprehensively define how mTOR signaling regulates skeletal muscle precursor cells with the hope of improving our ability to propagate these cells for therapeutic purposes. To achieve this, we are using mouse genetics to manipulate mTOR signaling in SMPs in vivo, then isolating and purifying the cells to determine the mechanism of how mTOR controls proliferation, differentiation, and muscle regeneration.

In Specific Aim 1, we use gain-of-function genetics to determine which SMP cell functions are driven by mTOR activity, while in Specific Aim 2 we use loss-of-function genetics to define the requirements for mTOR in muscle regeneration. Muscle stemcells have the potential to treat muscle degenerative diseases and may be the stem cell of origin in rhabdomyosarcoma, thus our studies will have broad impact towards understanding and treating multiple childhood diseases.

- **Adam Lacy-Hulbert, Ph.D.**

Assistant Professor
Massachusetts General Hospital

“Role of Alpha(v) Integrins in Establishment of Intestinal Immunity”

Key Words: Crohn's Disease, Colitis, Inflammation Immunity

Our long term research interests are in the regulation of immune responses, particularly in the intestine and other mucosal sites. We recently discovered a new mouse model of Inflammatory Bowel Disease (IBD) caused by genetic deletion of single adhesion molecule, alpha(v) integrin. Our understanding to date is that alpha(v) is required for the immune system to generate specific T cell populations that serve to down regulate immune responses to normal gut components (such as benign bacteria and food antigens) and also provide immune defense against disease-causing bacteria. Furthermore, we have found that this process occurs early in development in the mouse, before the age of weaning and colonization of the intestine by bacteria.

In this grant, we propose to understand how the intestinal immune system of young alpha(v) knockout mice differs from that control mice, and which of those differences go on to cause colitis in later life. We also aim to find out when in development these critical steps occur.

Successful completion of this work will lead to a greater understanding of the mechanisms by which immune regulation occurs in the intestine and will hopefully guide future treatment and prevention of childhood IBD

- **Paul Lerou, M.D.**

Instructor in Pediatrics
Children's Hospital Boston

“p53 Regulation in Human Pluripotent Stem Cells”

Key Words: Embryonic Stem Cell, Induced Pluripotency Stem Cell, p53, Cell Cycle

Human pluripotent stem (hPS) cells can be derived from human embryos or by reprogramming somatic cells via over-expressing defined pluripotency factors. These cells have enormous therapeutic potential as a source of cellular replacement therapy and can serve as a platform for in vitro study of disease and screening of therapeutic agents. The cell cycle of hPS cells differs significantly from that of somatic cells: nearly absent G1 phase, hyperphosphorylated retinoblastoma protein, constitutive cyclin E/A-CDK2 activity, and altered p53 activity. In somatic cells, such molecular alterations result in genomic instability and tumorigenesis, yet ES cells maintain genomic stability and retain the capacity to differentiate and contribute to normal organismal development. Recent data has shown that disabling p53 significantly increases the efficiency of reprogramming somatic cells to pluripotency, however, the impact on genomic stability and development potential of the resultant iPS cells is unclear. We hypothesize that although p53 regulation is altered in hPS, p53 protein plays an important role in maintaining genomic stability and the pluripotent state.

Aim1: Characterize the components of the p53-signaling network in human pluripotent stem cells. Although the p53 network has been extensively characterized in both somatic and cancer cells, this is not the case for hPS cells. RNA interference and well-characterized compounds will be used to interrogate this network in normally proliferating hPS cells and in response to DNA damage.

Aim 2: Use fixed and live-cell imaging techniques to characterize p53 dynamics. We have optimized culture conditions to image single hPS cells using immunofluorescence. We will perform quantitative image analysis to characterize p53 dynamics. We will also build a p53-fluorescent fusion protein reporter into hPS cell lines to perform quantitative live-cell imaging.

Our studies will translate into a better understanding of pluripotency and reprogramming thereby helping to realize the therapeutic potential of human pluripotent stem cells.

- **Jamie Maguire, Ph.D.**

Assistant Professor

Tufts University School of Medicine

“Impact of Maternal Depression on Offspring Development”

Key Words: Child Development, Postpartum Depression, Stress, Emotional Development, Cognitive Development

Postpartum depression is associated with deficits in child development. These studies have largely relied on correlations found in human studies due to the lack of useful animal models of postpartum depression. We have recently characterized a mouse model which exhibits abnormal postpartum behaviors, including depression-like behaviors, restricted to the postpartum period. We will utilize this model to test the hypothesis that maternal depression underlies the deficits in offspring development. We will examine anxiety, depression, and cognitive behaviors in offspring born to control mice and those born to mice exhibiting postpartum depression. In addition, we will perform cross-fostering experiments to determine if maternal depression directly influences child development. To determine how maternal depression may be transferred to the offspring, we will test the hypothesis that stress-induced steroid hormones negatively impact offspring development. Stress is a predicting factor for postpartum depression and elevated levels of stress-associated steroid hormones are associated with postpartum depression. To investigate if stress hormones may be passed from the mother to the offspring and mediate the negative impacts of postpartum depression on child development, the levels of the stress-related steroid hormone, corticosterone, will be compared between control mice and mice exhibiting postpartum depression. In addition, corticosterone levels will be measured in the offspring born to control mothers or mothers exhibiting postpartum depression. The impact of maternal stress on offspring behavior will be assessed in mice born to control mothers and mothers subjected to chronic ultramild stress. Children born to mothers with postpartum depression have deficits in emotional and cognitive development, increased incidence of violent crime, depression, drug abuse, and suicide. Insight into how these negative aspects are transferred from mother to offspring will be relevant to all these negative issues regarding child development.