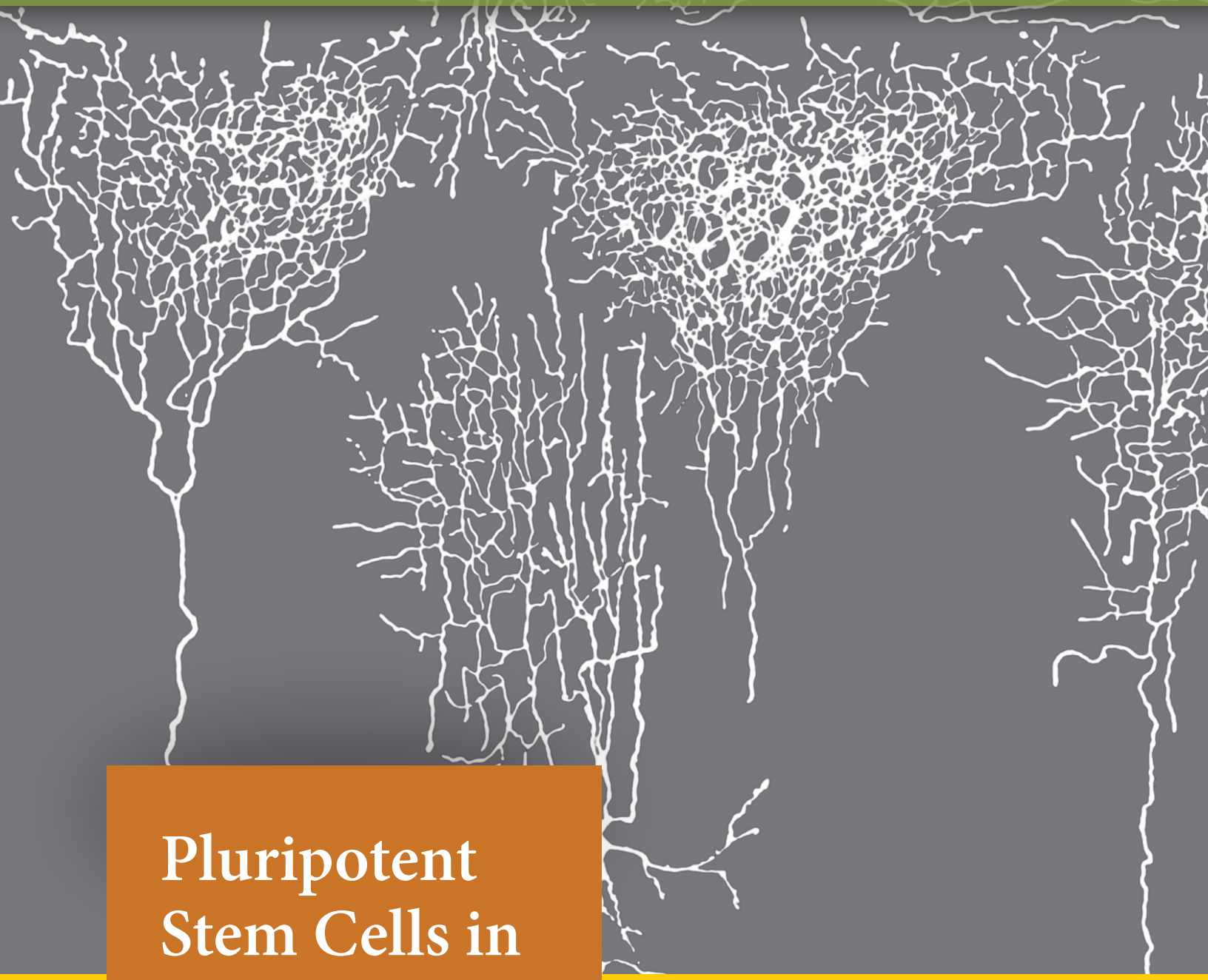
National Institute of Neurological Disorders and Stroke



Pluripotent Stem Cells in Neuroscience:

Research and Application Symposium

One Day Symposium

October 26, 2015

John Edward Porter Neuroscience Research Center

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Purpose:

NINDS presents a symposium intended to bring together the intramural neuroscience community interested in using pluripotent stem cells in their research or other applications. It will highlight some of the research being performed on campus with the goal of fostering new collaborations intramurally. The inclusion of distinguished extramural leaders in the neuroscience field will also stimulate new ideas and discussion.

About the Organizers:

The NIH Stem Cell Unit was established in 2003 to characterize and study the human embryonic stem cell lines available at that time. Their mandate has expanded with the advent of somatic cell reprogramming to include induced pluripotent stem cells. They remain a resource, available to the NIH community, for advice and assistance on relevant projects.

Kapil Bharti, Ph.D. is an Earl Stadtman Tenure-Track Investigator and the head of Ocular and Stem Cells Translational Research unit at NEI. His current work involves developing cell-based therapy for retinal degenerative diseases using induced pluripotent stem cell technology.

Pamela Gehron Robey, Ph.D., is chief of the Skeletal Biology Section, and chief of the Craniofacial and Skeletal Diseases Branch of the NIDCR, and Acting Scientific Director of the NIH Stem Cell Unit. She has worked in the area of bone and stem cell biology for over 20 years, which includes basic, translational and clinical studies.

Contact:

Barbara Mallon, D.Phil.
Technical Director, NIH Stem Cell Unit
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mallonb@mail.nih.gov

Front and back cover:

Infant motor cortex. Santiago Ramon y Cajal (Google Books)

Inside front and back cover:

Phase image of neurons differentiated from iPSCs. Barbara Mallon (NIH SCU)

Agenda

8:30 a.m. –8:45 a.m.

Opening Remarks

Pamela G. Robey, Acting Director, NIH Stem Cell Unit

Kapil Bharti, NEI

8:45 a.m. –10:30 a.m.

PSCs and Neuropsychiatric Disorders – *F. McMahon (Chair)*

Avindra Nath, NINDS	*Role of Endogenous Retroviral Elements in the Proliferation and Differentiation of Stem Cells*
Kristen Brennand, Mount Sinai, NY	*Modeling Predisposition to Schizophrenia using hiPSCs*
Francis McMahon, NIMH	*Induced Pluripotent Stem Cell Models of Major Mental Illness*

10:30-10:45 a.m.

Coffee & Posters

10:45 a.m. –12:30 p.m.

Neurological Disorders – *E. Sidransky (Chair)*

Kevin Eggan, Harvard University, MA	*Using Stem Cells to Study the Pathways to Motor Neuron Degeneration in ALS*
Christopher Grunseich, NINDS	*RNA Processing in Motor Neuron Disease*
Elma Aflaki, NHGRI	*A New Glucocerebrosidase Chaperone Reduces α-Synuclein in iPSC-Derived Dopaminergic Neurons from Patients with Gaucher Disease and Parkinsonism*

12:30 p.m. –1:30 p.m.

Lunch

Agenda

1:30 p.m. –2:15 p.m.

Poster Session

2:15 p.m. –3:25 p.m.

Clinical Translation – *K. Bharti (Chair)*

Edward Wirth III, Asterias Biotherapeutics, CA	*Initial Clinical Trials of hESC-Derived Oligodendrocyte Progenitor Cells in Subacute Spinal Cord Injury*
Kapil Bharti, NEI	*Induced to Cure: Engineering iPS Cell Derived RPE*

3:25 p.m. –3:45 p.m.

Coffee & Posters

3:45 p.m. –5:30 p.m.

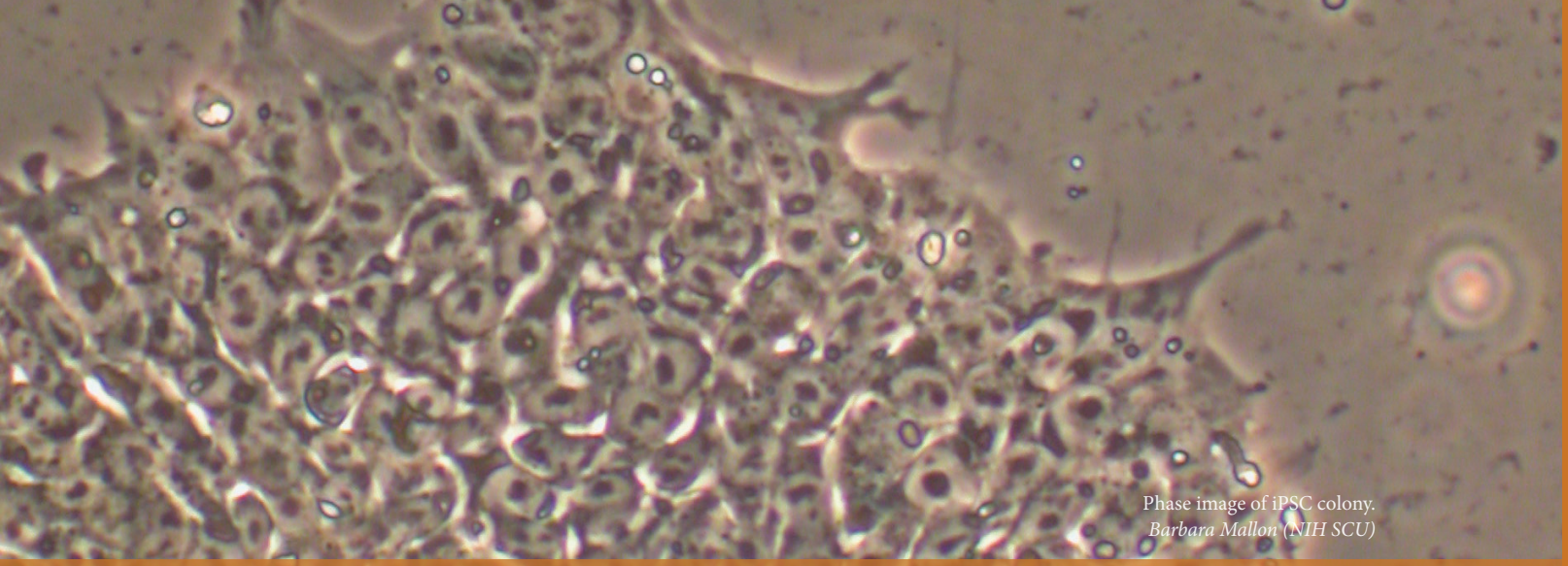
PSC Biology and Tools for Study – *P. Robey (Chair)*

Ron McKay, Lieber Institute for Brain Development, MD	*Defining the Origins of Variation in Human Pluripotent Stem Cells*
Steven Finkbeiner, Gladstone Institute, CA	*Harnessing Patient Stem Cells to Understand Disease and Find Therapies*
Ilyas Singeç, NCATS	*The Next Phase of the NIH Center for Regenerative Medicine (NCRM): Leveraging Stem Cell Biology for Drug Discovery and Clinical Applications*

5:30 p.m.

Closing Remarks

Barbara Mallon, NINDS



Phase image of iPSC colony.
Barbara Mallon (NIH SCU)

Speakers

Elma Aflaki, Ph.D.

Clinical Research Fellow, National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

Dr. Elma Aflaki is a Research Fellow with Dr. Ellen Sidransky in the Molecular Neurogenetics Section of the National Human Genome Research Institute. She received both her B.Sc. and an M.Sc. in molecular genetics from Tehran University, Iran and graduated from the Medical University of Graz, Austria with a PhD in molecular medicine in 2011. Her research has focused on the lipid metabolism of macrophages and their role in the regulation of inflammation. Based on the importance of macrophages in Gaucher disease (the only cell types which manifest accumulation) and her background in lipid metabolism and inflammation of these cells, she joined Dr. Sidranky's lab as visiting fellow to study the cell biology of Gaucher Disease and the effect of small molecules in Gaucher macrophage functions such as phagocytosis, efferocytosis and chemotaxis. Dr. Aflaki is also investigating the differentiation of pluripotent stem cells into macrophages and dopaminergic neurons from Gaucher and Parkinson patients to investigate the association between these diseases and to study the effect of small molecules as candidate drug for Gaucher and Parkinson. In 2014, she was awarded a NHGRI Intramural Research Award for scientific accomplishment.

Kapil Bharti, Ph.D.

Earl Stadtman Tenure-Track Investigator, Unit on Ocular and Stem Cell Translational Research, National Eye Institute, National Institutes of Health, Bethesda, MD

Dr. Kapil Bharti holds a bachelor's degree in Biophysics from the Panjab University in Chandigarh, India, a master's degree in biotechnology from the M.S. Rao University in Baroda, India, and a diploma in molecular cell biology from the Johann Wolfgang Goethe University in Frankfurt, Germany. He obtained his Ph.D. from the same institution, graduating summa cum laude. His Ph.D. work involved research in the areas of heat stress, chaperones, and epigenetics. He did his postdoc at the National Institutes of Health, where he published numerous papers in the areas of transcription regulation, pigment cell biology, and developmental biology of the eye. Dr. Bharti has successfully gotten grants in three out of three cycles from the NIH Center for Regenerative Medicine. He has won several awards, including, most recently, being a finalist in the prestigious trans-NIH Earl Stadtman Tenure-Track Symposium. His lab was recently awarded the only Intramural Common

Fund grant to develop a phase I Investigational New Drug (IND) for Autologous induced pluripotent stem cell derived retinal pigment epithelium tissue. His current work as the head of the Unit on Ocular and Stem Cell Translational Research involves developing cell-based therapy for retinal degenerative diseases using induced pluripotent stem cell technology.

Kristen Brennand, Ph.D.

Assistant Professor, Icahn School of Medicine at Mount Sinai, New York, NY

Kristen Brennand, PhD is an Assistant Professor of Psychiatry at the Icahn School of Medicine at Mount Sinai, in New York, New York. She trained in developmental and stem cell biology with Dr. Douglas Melton during her graduate studies at Harvard University and in neurobiology during postdoctoral research with Dr. Fred Gage at the Salk Institute for Biological Studies. By combining expertise in stem cell biology and neurobiology, she has pioneered a new approach by which to study psychiatric disease. The focus of her research is schizophrenia, a debilitating psychiatric disorder for which there exists no cure. The genetic mechanisms responsible for this disorder are not well understood, the precise cell type affected remains unknown and few defining characteristics of diseased cells have been identified, in part due to the lack of live brain tissue for study. Kristen is working to understand how neurons from patients with schizophrenia differ from those from healthy controls. Specifically, she obtains skin samples from patients, which are then reprogrammed into induced pluripotent stem cells, and subsequently differentiated into human neurons. The goal of her research is to better understand the genetic and cellular mechanisms contributing to schizophrenia, in the hope that this may one day lead to new potential therapies. Dr. Brennand has secured funding for her work from the National Institutes of Health, the New York Stem Cell Foundation and the Brain and Behavior Research Foundation.

Kevin Eggan, Ph.D.

Professor, Stem Cell and Regenerative Biology, Harvard University; Principal Investigator, Harvard Stem Cell Institute; Early Career Scientist, Howard Hughes Medical Institute

Dr. Eggan is currently a Professor in the Department of Stem Cell and Regenerative Biology at Harvard University as well as Director of the Stem Cell Program at the Stanley Center for Psychiatric Research at the Broad Institute. He completed his PhD studies with Dr. Rudolf Jaenisch at the Massachusetts Institute of Technology in 2002 and was appointed junior fellow at Harvard University in 2003. He became assistant professor of Molecular & Cellular Biology at the Stem Cell Institute in 2005 and has garnered a number of high profile awards, including the MacArthur Foundation "Genius Grant" in 2006. In 2009, Dr. Eggan was selected as one of 50 Howard Hughes Medical Institute Early Career Scientists. He has made fundamental contributions to the fields of stem cell biology and cellular reprogramming including nuclear transfer studies, conducted during training, which challenged preconceived notions concerning the limits of cellular plasticity. His own lab then became the first to demonstrate that human somatic cells could be reprogrammed to an embryonic stem (ES) cell state. This work has been cited as an inspiration for the discovery of factors used to generate induced pluripotent stem cells (iPSCs). His lab was also first to generate patient-specific iPSCs and use them to produce the cell type affected in that individual. As these particular patients suffered from ALS, his lab has since explored stem cells as a renewable source of motor neurons for studying mechanisms leading to neural degeneration. Using this approach, they have found that astrocytes are important non-cell autonomous contributors to motor neuron degeneration in ALS, discovered new mechanisms that lead to motor neuron degeneration, and have identified a candidate ALS therapeutic. In his role as Director of the Stem Cell Program at the Stanley Center for Psychiatric Research at the Broad Institute, Dr. Eggan is leading a group of scientists to expand the platform to increase reproducibility of stem cell and reprogramming technologies with the ultimate goal of improving understanding and treatment of psychiatric diseases.

Steven Finkbeiner, M.D., Ph.D.

Professor, Departments of Neurology and Physiology, UCSF; Senior Investigator and Associate Director, Gladstone Institute of Neurological Disease; Director, Taube/Koret Center for Neurodegenerative Disease Research

Dr. Finkbeiner is the Associate Director and Senior Investigator at the Gladstone Institute of Neurological Disease (GIND), the Director of the Taube/Koret Center for Neurodegenerative Disease Research and the Hellman Family Foundation Alzheimer's Disease Research Program. He is also a Professor of Neurology and Physiology at the University of California, San Francisco (UCSF). In 1986, Dr. Finkbeiner earned a bachelor's degree from Wheaton College. He earned both an MD and a PhD in neuroscience from Yale University in 1991. He completed an internship in internal medicine and chief residency in neurology at UCSF, followed by a research fellowship at Harvard Medical School, and in 1999 he was one of the first investigators at GIND. Dr. Finkbeiner is a fellow of the American Neurological Association, and is a member of many scientific and professional societies, including the Society for Neuroscience, the Society for Cell Biology and the Biophysical Society. He is active in graduate training and is a member of the Neuroscience, Biomedical Sciences and Medical Scientist Training Programs at UCSF. Dr. Finkbeiner studies the molecular mechanisms that are responsible for learning, memory and neurodegeneration. A better understanding of the mechanisms that control memory formation and neuronal survival will yield crucial insights into the pathogenesis of neurodegenerative diseases and the memory disorders that often characterize them. A major focus of his work in the area of neurodegenerative disease has been the role of protein dyshomeostasis in Huntington's disease, Parkinson's disease, ALS, and Frontotemporal dementia.

Christopher Grunseich, M.D.

Clinical Research Fellow, National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

Christopher Grunseich, MD is a senior clinical fellow in the Neurogenetics Branch/NINDS in the lab of Dr. Kenneth Fischbeck. He received his MD from SUNY Stony Brook where he studied gene regulation during neuronal development and the role of astrocytes in Rett Syndrome as an HHMI Fellow with Dr. Gail Mandel. He completed his residency in Neurology at Georgetown University in 2010, and has since been studying disease modeling and mechanisms using patient derived iPSC. His work has focused on understanding tissue specific disease disturbances in two types of motor neuron disease: Spinal and Bulbar Muscular Atrophy (SBMA) and Amyotrophic Lateral Sclerosis, type 4 (ALS4). He is using iPSC differentiated tissues to explore the disease mechanism and identify targets that can be used in the development of treatment for these diseases.

Ron McKay, Ph.D.

Director for Basic Sciences, Lieber Institute for Brain Development, Baltimore, MD

Ron McKay, Ph.D is the Lieber Institute for Brain Development Director for Basic Sciences. Before joining the Lieber Institute, Dr. McKay was Chief of the Laboratory of Cellular Neurobiology of the National Institute of Neurological Disorders and Stroke (NINDS). Dr. McKay received a B.Sc. in 1971 and a Ph.D. in 1974 from the University of Edinburgh. His postdoctoral training was at the University of Oxford. In Edinburgh and Oxford, he contributed to the earliest work showing that the tools of molecular biology would make a major contribution to human genetics. In 1978, he moved to Cold Spring Harbor Laboratory. At Cold Spring Harbor, he was the first to show that specific DNA-protein complexes could be analyzed with antibodies, and pioneered the field of molecular neuroscience. Joining the MIT faculty in 1984, Dr. McKay identified neural stem cells as a tool to study brain development and function. In 1993, he joined the NIH as Chief of the Laboratory of Molecular Biology at NINDS. His laboratory at the Lieber Institute studies pluripotent and somatic stem cells with a particular interest in the development of the nervous system. His research

is focused on using the biology of stem cells to understand the genetic basis of human disease and to regenerate injured tissue. He is a founding board member of the International Society of Stem Cell Research. He has served on Scientific Advisory Boards of commercial and academic programs across the world. He is the recipient of the Ernst Schering Prize and the Robert Menzies, and Max Delbrück Medals

Francis J. McMahon, M.D.

Chief, Human Genetics Branch and Genetic Basis of Mood and Anxiety Disorders Section, National Institute of Mental Health Intramural Research Program, National Institutes of Health, Bethesda, MD

Dr. McMahon received a B.A. in Biology from the University of Pennsylvania in 1982 and an M.D. from Johns Hopkins in 1987, where he also completed a medical internship, a residency in adult psychiatry, and a post-doctoral fellowship in genetics. In 1998, he moved to the University of Chicago, where he continued his research into the genetics of bipolar disorder while serving as Medical Director of the electroconvulsive therapy clinic. In 2002, he joined the Mood and Anxiety Disorders Program of the National Institute of Mental Health (NIMH) Intramural Research Program as Chief of its genetics unit. In 2010, he was promoted to Senior Investigator and Chief of the Human Genetics Branch. He is also a visiting Professor of Psychiatry at the Johns Hopkins University School of Medicine.

Dr. McMahon's research is aimed at discovering and characterizing genes involved in mood and anxiety disorders, such as bipolar disorder, major depression, and panic disorder. In addition to genome wide association and next generation sequencing studies, his laboratory is involved in functional genomics studies using RNA-seq and induced pluripotent stem cell-based models. The long-term objective of his research is to develop a complete picture of the genetic architecture of mood and anxiety disorders, so that better methods of diagnosis and treatment can be developed.

Dr. McMahon is also active outside of NIMH. He is President of the International Society of Psychiatric Genetics, and a member of the American College of Neuropsychopharmacology, the Society of Biological Psychiatry, and the American Society of Human Genetics. He serves on the editorial boards of Biological Psychiatry and International Review of Psychiatry. He serves as a scientific advisor for the American Society for the Prevention of Suicide, and the Rutgers University Cell & DNA Repository. He has authored over 175 scientific reports and textbook chapters.

Dr. McMahon has received awards for his work from the National Alliance for Research on Schizophrenia and Depression (NARSAD), the National Alliance for the Mentally Ill, NIMH, and The Edward F. Mallinckrodt Foundation. His work in genome wide association studies has been recognized with the NIH Director's Award for Significant Achievement.

Avindra Nath, M.D.

Chief, Section of Infections of the Nervous System; Director, Translational Neuroscience Center; National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

Dr. Avindra Nath is the Clinical Director of the National Institute of Neurological Diseases and Stroke and the Chief of the Section of Neurological Infections. He is also the President of the International Society of Neurovirology. His research is focused on the pathophysiology and treatment of neurological complications of HIV infection including opportunistic infections. In particular, his laboratory is studying how HIV enters the nervous system and establishes a reservoir in glial cells and maintains latency in these cells. Further his group is studying the mechanisms by which these HIV infected glial cells lead to neurodegeneration. Through interactions with the Translational Neuroscience Center they are developing therapeutic approaches to block the formation of the reservoirs, preventing viral activation in the glial cells and developing ways of accelerating the turnover of the

infected cells in the brain. His laboratory also closely interacts with investigators in other institutes to establish a cohort of HIV infected patients where the pathophysiology of the infection can be studied and these therapeutic approaches will be tested. In recent years, his group has begun to explore the role of endogenous retroviruses in neurological Diseases. In particular they are interested in determining how these viruses get activated in the brain, if they can be transmitted and if they can cause neurodegeneration or neuroinflammation. In 2012, he received the Pioneer award from the International Society of Neurovirology.

Ilyas Singeç, M.D., Ph.D.

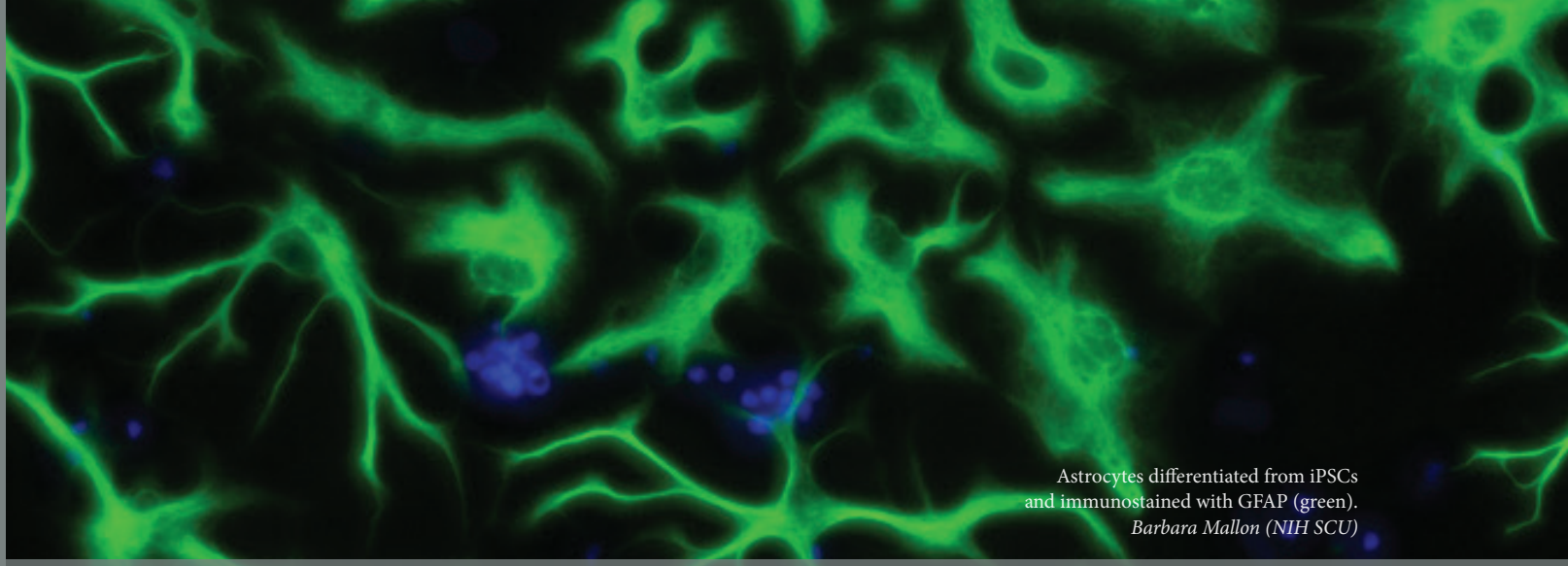
Head, NIH Stem Cell Technology Facility, Center for Regenerative Medicine (NIH CRM), National Center for Advancing Translational Sciences (NCATS), National Institutes of Health, Bethesda, MD

Dr. Ilyas Singeç is the Head of the NIH Stem Cell Technology Facility and leads the new phase of the Center for Regenerative Medicine, which is supported by the Common Fund and located at the National Center for Advancing Translational Sciences (NCATS). Dr. Singec completed his MD and doctorate in neurobiology (*summa cum laude*) at the Albert-Ludwigs-University in Freiburg, Germany. After clinical residency in Neuropathology, he conducted postdoctoral work at NIH/NINDS and the Sanford Burnham Prebys (SBP) Medical Discovery Institute. As a Lab Head at Pfizer, he was the biology lead of a preclinical drug discovery program and developed human stem cell-based assays for new target identification & validation. In his earlier scientific work, Dr. Singeç characterized the molecular and cellular diversity of the normal and diseased brain by using novel biomarkers for the detection of rare neuronal cell types as well as disease mechanisms (e.g. aberrant synaptic plasticity in temporal lobe epilepsy). He then spearheaded important studies in the neural stem cell field and was the first to demonstrate the limitations of the widely used neurosphere assay for clonal analysis. He also invented a novel slice co-culture model by connecting the rostral migratory stream (RMS) to the hippocampus. These studies demonstrated that RMS neuroblasts, typically destined to be become olfactory interneurons, can be reprogrammed into CA1 pyramidal neurons by environmental cues of the host tissue. Dr. Singeç's recent work has focused on the generation and use of patient- and disease-specific induced pluripotent stem cells (iPSCs). At the Sanford Burnham Prebys (SBP) Medical Discovery Institute he served as the Director of Cell Reprogramming and pioneered the large-scale production of new iPSC lines. In addition, having developed a rapid 6-day neural induction method, he established the largest quantitative proteomic and phosphoproteomic datasets of human pluripotent cells and directly compared them to their earliest definitive neural stem cells. This work also led to the identification and functional characterization of midkine, a new autocrine/paracrine factor controlling neural induction. His current translational research interests include genetics-based disease modeling of neurological and psychiatric disorders, precise characterization of cell type identities, and developing novel approaches to elucidate pluripotency and controlled cellular differentiation by using integrated and scalable systems biology methods. Dr. Singeç has been the recipient of several scientific awards and fellowships from Pfizer, Merck, International Bipolar Foundation, German Research Council (DFG), California Institute for Regenerative Medicine (CIRM), and NIH.

Edward D. Wirth, III, M.D., Ph.D.

Chief Medical Officer; Asterias Biotherapeutics, Inc., Menlo Park, CA

Edward D. Wirth, III, M.D., Ph.D., became our Chief Translational Officer during March 2013 after serving as Chief Science Officer at InVivo Therapeutics Corporation from 2011 to 2012. From 2004 to 2011, Dr. Wirth served as Medical Director for Regenerative Medicine at Geron Corporation, where he led the world's first clinical trial of a hES cell-derived product, GRNOPC1 in patients with subacute spinal cord injuries. Dr. Wirth held academic appointments at Rush-Presbyterian St. Luke's Medical Center and at the University of Chicago from 2002 to 2004, and was a member of the faculty of the University of Florida from 1996 to 2002. Dr. Wirth received his Ph.D. and M.D. from the University of Florida in 1992 and 1994, respectively.



Astrocytes differentiated from iPSCs and immunostained with GFAP (green). *Barbara Mallon (NIH SCU)*

Speaker Abstracts

Role of Human Endogenous Retroviruses in Stem Cell Differentiation and Neural Development

Tongguang Wang and Avindra Nath

National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

Human endogenous retrovirus (HERV) gene sequences occupy almost 7% of the human genome. Although once considered "junk DNA", some of the genes may still be active in certain physiological or pathological conditions and play important roles in human development and disease pathogenesis. Retroelements are relatively active in the embryo and pluripotent stem cells, likely due to their specific epigenetic status. In embryonic stem cells (ES) or induced pluripotent stem cells (iPSC), there are increased expressions of HERV-H and certain HERV-K components, which decrease during cell differentiation. Besides, HERV-H may regulate the stemness of these cells by producing long non-coding RNA (LncRNA). We studied the expression and function of HERV-K components in pluripotent stem cells and during the process of neuronal differentiation. We found that HERV-K components, especially cell membrane envelope (Env) expression, were increased after iPSC generation compared to the source cells such as fibroblasts and CD34 cells. The expression of Env decreased dramatically after iPSC were differentiated to neural stem cells and neurons. Interestingly, treatment with antibodies and siRNA against Env resulted in fewer stem cell colonies. When Env expression was blocked by siRNA, there were significant morphological changes in iPSC. Further, Env was co-immuoprecipitated with CD98HC, a protein that plays an important role in cell adhesion and stem cell maintenance. Western-blot assays showed that blocking Env using siRNA resulted in decrease in CD98HC expression. These results indicate that Herv-K Env is expressed in pluripotent stem cells and decreases during neural cell differentiation. It may play an important physiological role in maintaining the pluripotent stem cells by interacting and regulating other factors expressed on stem cells such as CD98HC. Its possible activation in human embryo may provide a unique contribution to human brain development.

Modeling Predisposition to Schizophrenia Using hiPSCs

Kristen Brennand, Ph.D.

Icahn School of Medicine at Mount Sinai, New York, NY

Schizophrenia (SZ) is a debilitating psychiatric disorder. While 1.1% of the population suffers from SZ, the molecular mechanisms underlying the disease state remain unclear; moreover, although postmortem studies

have revealed reduced neuron size and spine density in SZ brain tissue, the molecular mechanisms underlying the disease state remain unclear. Though its characteristic symptoms typically appear late in adolescence, SZ is believed to result from abnormal neurodevelopmental processes that begin years before the onset of symptoms. We previously reprogrammed fibroblasts from SZ patients into human induced pluripotent stem cells (hiPSCs) and subsequently differentiated these disorder-specific hiPSCs into neural progenitor cells (NPCs) and neurons. We have found that SZ hiPSC NPCs show evidence of aberrant migration, elevated WNT signaling, increased oxidative stress and higher global protein synthesis, while SZ hiPSC neurons exhibit decreased neurite number, reduced synaptic maturation and reduced synaptic activity. Although hiPSC-derived neurons most resemble human fetal brain tissue and presumably best model disease predisposition, there has been good concordance between findings from our hiPSC studies and established mouse models of SZ. Recently, we developed novel methodologies to accelerate the maturation of functional glutamatergic neurons, and so are conducting genome wide expression analysis (RNAseq) of populations of NPCs and neurons derived from an expanded and clinically characterized cohort of childhood onset schizophrenia patients. Ultimately, we will manipulate, either causal regulators of aberrant gene expression in SZ neurons, in the hope that restoring normal gene expression of key genes will be sufficient to restore normal synaptic morphology and neuronal function in diseased neurons.

Induced Pluripotent Stem Cell Models of Major Mental Illness

Francis McMahon, M.D.

National Institute of Mental Health, National Institutes of Health, Bethesda, MD

Genetic studies have identified several risk variants involved in major mental illnesses, including common variants that exert small effects and rare copy number variants that exert larger effects on risk. Despite this progress, the biological characterization of risk variants in multigenic disorders remains a substantial challenge, impeding translation of genetic study results into better methods of diagnosis and treatment. Direct analyses of the impact of risk variants on gene expression in the brain has been limited by a reliance on post-mortem studies as the main means of accessing appropriate cells from affected patients. This is beginning to change thanks to the advent of iPSC technology and new tools that enable the genetic manipulation of patient-derived cells in vitro. iPSC are particularly valuable for the investigation of multigenic disorders, since the cells capture the full complement of risk alleles within the same genetic background as the individual from whom they derive. We are using human iPSC and their neuronal derivatives to characterize common and rare genetic variants contributing to bipolar disorder, an incurable mental illness that causes considerable morbidity and mortality. With this system, we can easily measure relationships between genotype and gene expression, search for effects of known risk variants on cellular phenotypes, and test the impact of commonly used therapeutic agents. Some specific examples will be presented. Neural cell models based on iPSC offer a relatively straightforward approach to the biologic characterization of genetic variants associated with neuropsychiatric disorders that may ultimately help illuminate therapeutic mechanisms.

Using Stem Cells to Study the Pathways to Motor Neuron Degeneration in ALS

Kevin Eggan, Ph.D.

Harvard University, Cambridge, MA

In my laboratory, we pursue two interlocking areas of investigation: the basic biology of stem cell programming and reprogramming, as well as the application of the resulting technologies to studies of the neuromuscular system and the diseases that affect it.

A fundamental understanding of how a cell's identity is determined during differentiation and how it can in turn be manipulated experimentally is a central goal of developmental biology, one with substantial ramifications for biomedicine. We study both the differentiation of embryonic stem cells into the neural lineage and the reprogramming of commonly available differentiated cell types, such as fibroblasts, into either pluripotent stem cells or cells of therapeutic interest, such as spinal motor neurons. To study differentiation and dedifferentiation, we

employ a variety of approaches, including stem cell differentiation, nuclear transfer, and defined reprogramming strategies using known transcriptional regulators and novel small-molecule compounds.

A number of devastating diseases, including amyotrophic lateral sclerosis (ALS) and spinal muscular atrophy (SMA), specifically affect the neuromuscular system. Little is known concerning the molecular pathology underlying these conditions, partly because it has been impossible to access significant quantities of the disease-affected cell, the spinal motor neuron. With recent advances in stem cell and reprogramming biology, we can now produce billions of spinal motor neurons with control and diseased genotypes. We use this new resource to design in vitro disease models for both mechanistic studies and for the discovery of novel small-molecule therapeutics.

RNA Processing in Motor Neuron Disease

Christopher Grunseich, M.D.

National Institute of Neurological Disorders and Stroke, National Institutes of Health, Bethesda, MD

Dysregulation of RNA processing plays a key role in motor neuron disorder; however, the underlying mechanisms and the particular vulnerability of motor neurons are largely unknown. ALS4 is a degenerative disease caused by autosomal dominant mutations in the RNA-DNA helicase, senataxin. The disease is characterized by slowly progressive weakness, with signs of both upper and lower motor neuron involvement. Senataxin resolves RNA/DNA hybrids (R-loops), which form during transcription as nascent transcripts hybridize with the unwound DNA duplex. These transcriptional based R-loops have been associated with alterations in RNA processing, genomic instability, and trinucleotide repeat sequences. To understand how changes in R-loop biology affect motor neuron function, and to use the resulting knowledge for therapeutic development, we are carrying out clinical and molecular phenotyping of individuals in a large Maryland family with ALS4 due to a L489S senataxin mutation. To study RNA processing, we collected various cells and generated induced pluripotent stem cells (iPSC) from ALS4 patients and controls. CRISPR-Cas engineering was used to create iPS cells with and without the L389S mutation in senataxin. We observed that there is a reduction in the abundance of R-loops in patient cells by immunoprecipitating using the S9.6 antibody for R-loop identification. We also studied nascent RNA using PRO-seq to provide sensitive detection of those regions with pauses in transcription and understand how these changes are related to dysfunction. In this presentation, I will discuss results which show how R-loop biology is disrupted in ALS4 and how this disruption may be contributing to the disease.

A New Glucocerebrosidase Chaperone Reduces α-Synuclein in iPSC-Derived Dopaminergic Neurons from Patients with Gaucher Disease and Parkinsonism

Elma Aflaki, Ph.D.

National Human Genome Research Institute, National Institutes of Health, Bethesda, MD

The ability to induce pluripotent stem cells to generate dopaminergic neurons from patients provides an important tool to study the pathology and treatment of Parkinson disease. Among the genes implicated in Parkinson disease, the gene encoding glucocerebrosidase, GBA1, mutated in the lysosomal disorder Gaucher disease, confers the highest risk. In order to evaluate the impact of glucocerebrosidase on parkinsonism, we generated macrophages and dopaminergic neurons from four patients with type 1 Gaucher disease, two with and two without parkinsonism, including a sibling pair discordant for Parkinson disease, as well as one patient with type 2 (acute neuronopathic) Gaucher disease. Studies of catecholamine metabolites indicated that mDA neurons from patients with type 2 and type 1 Gaucher disease with parkinsonism had reduced dopamine storage and dopamine transporter reuptake. These cells exhibited decreased glucocerebrosidase activity and stored the glycolipid substrates glucosylceramide and glucosylsphingosine. Levels of α-synuclein, a protein present as aggregates in Parkinson disease and related synucleinopathies, were selectively elevated in neurons from the patients with parkinsonism or type 2 Gaucher disease. The cells were then treated with NCGC607, an optimized small molecule non-inhibitory chaperone of glucocerebrosidase identified after high through-put screening and medicinal chemistry structure optimization.

This compound successfully restored glucocerebrosidase activity and protein levels and reduced glycolipid storage in both iPSC-macrophages and dopaminergic neurons, indicating that this compound class has potential for correcting the Gaucher phenotype including neuronopathic involvement. In addition, NCGC607 reduced α-synuclein levels in dopaminerigic neurons from the patients with parkinsonism, suggesting that non-inhibitory small molecule chaperones of glucocerebrosidase might have utility for the treatment of Parkinson disease.

Initial Clinical Trials of hESC-Derived Oligodendrocyte Progenitor Cells in Subacute Spinal Cord Injury

Edward D. Wirth, III, M.D., Ph.D.

Asterias Biotherapeutics, Inc., Menlo Park, California USA

Preclinical studies of AST-OPC1 (formerly GRNOPC1) in animal models of traumatic SCI have shown that these cells survive, migrate throughout the injury site, and improve locomotor function. Therefore, a phase 1 clinical trial was initiated to assess the safety of AST-OPC1 in patients with neurologically-complete T3-T11 thoracic SCI. Five subjects received a low dose of 2 x 10^6 AST-OPC1 cells between 7 and 14 days following their injury. The cells were administered via a single injection into the spinal cord lesion using a dedicated syringe positioning device. Subjects received a low dose of tacrolimus immunosuppression for 46 days, which was then tapered and discontinued at day 60. Subjects were followed for 1 year under the main study protocol and are being followed for an additional 14 years under a long-term follow up protocol. To date, all five patients have been followed for over 3 years. There have been no serious adverse events related to AST-OPC1, tacrolimus, or the injection procedure. Serial MRI scans indicate that lesion cavity formation at the AST-OPC1 injection sites was substantially reduced through 3 years of follow up in 4 of 5 subjects. There were no unexpected changes in neurological function. The data to date suggest that AST-OPC1 can be safely administered to patients in the subacute period after thoracic SCI.

The next phase of development will be a Phase 1/2a trial to evaluate the safety and activity of escalating doses of AST-OPC1 in patients with subacute sensorimotor complete cervical SCI. The first participant in this trial received AST-OPC1 in June, 2015 and enrollment of the initial cohort of three participants was completed in August, 2015. Participants in this cohort received a low dose of 2 x 10^6 AST-OPC1 cells between 14 and 30 days following their injury. There were no intraoperative complications in this cohort and no serious adverse events have been reported to date. Enrollment of the second dose cohort is expected to begin following Data Monitoring Committee review of the 30-day post-injection safety data from all three participants. The second cohort will enroll five patients who will receive 10 million AST-OPC1 cells.

Induced to Cure: Engineering iPS Cell Derived RPE Scaffolds to Treat Degenerative Eye Diseases

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The recent successful phase I trial using embryonic stem (ES) cell derived retinal pigment epithelium (RPE) has provided hope for a cure for degenerative eye diseases. Induced pluripotent stem (iPS) cells are an alternate and an autologous source of stem cells potentially with fewer immune-challenges as compared to ES cells. Using a developmentally guided differentiation protocol we have developed fully polarized RPE tissue from iPS cells. The RPE monolayer along with its secreted ECM and a biodegradable scaffold form a tissue that well mimics the native tissue in structural and functional properties. This tissue has been functionally authenticated *in vitro* for its ability to perform several key RPE functions like phagocytosis of photoreceptor outer segments, ability to transport water from apical towards basal sides, and ability to secrete cytokines in a polarized fashion. Currently, we are testing the safety and the efficacy of this tissue in animal models. We have begun Phase I Investigational New Drug (IND) enabling studies with the goal to transplant autologous iPS cell derived RPE in patients in advanced Geographic

Atrophy stage of age-related macular degeneration (AMD), one of the leading blinding diseases in the US. Our work will provide a potential personalized cell therapy for AMD patients.

Defining the Origins of Variation in Human Pluripotent Stem Cells

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Lieber Institute for Brain Development, Baltimore, MD

Pluripotent stem cells and their lineage-committed derivatives are poised to revolutionize biomedicine. Efforts to characterize these cells have shown that their differentiation potential can vary widely. The sources of this variability across human stem cell lines have been elusive without tools to control the early transitions that lay out the body plan of a new individual. We report a system where human stem cells spontaneously self-organize to form an epithelium with developmentally distinct zones primed to generate anterior or posterior fates along the primary embryonic axis. This system defined stable differences between cell lines in the earliest patterning events and in transcriptional signatures specific to individual humans. Reconstructing the earliest stages of development in the laboratory opens a systematic strategy to map the origins of variation in human development.

Harnessing Patient Stem Cells to Understand Disease and Find Therapies

Steven Finkbeiner, M.D., Ph.D.

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This talk will describe ongoing efforts to use patient-derived induced pluripotent stem cells (iPSCs) to develop models of Huntington's disease, Parkinson's disease, amyotrophic lateral sclerosis, frontotemporal dementia, and Alzheimer's disease. Strategies for overcoming some of the challenges associated with using iPSCs will be discussed, including the application of high throughput single cell analysis and machine learning. We will explain how we integrate iPSCs into our work to find therapeutic targets and small molecule therapeutics with reference to family-based whole genome analysis and a particular emphasis on protein homeostasis and the protein clearance pathway autophagy.

The Next Phase of the NIH Center for Regenerative Medicine (NCRM): Leveraging Stem Cell Biology for Drug Discovery and Clinical Applications

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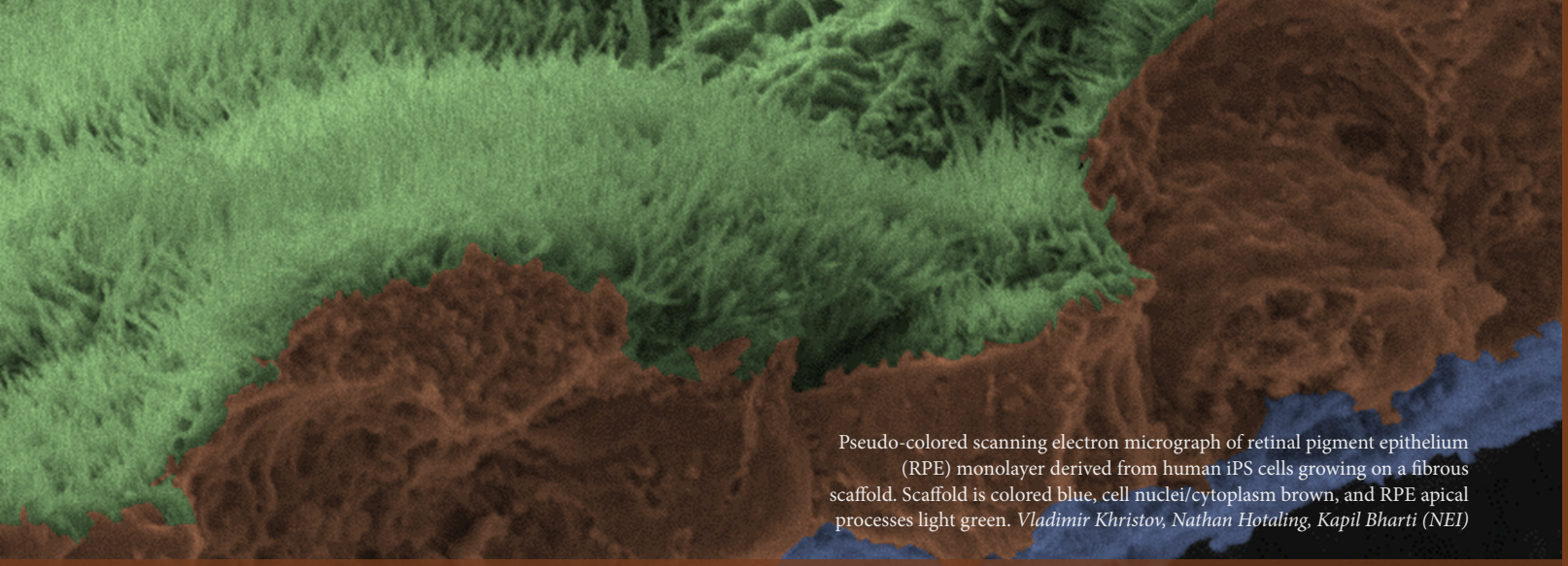
Pluripotent stem cells hold great promise to develop novel therapies for various intractable human diseases. However, to fulfill this promise the stem cell field has to progress more efficiently from basic discovery to therapeutic applications. Currently, few robust tools, technologies, protocols, and paradigms exist that allow researchers to reproducibly and efficiently generate relevant cell types at desirable quantity, purity, and functionality. Multiple needs analyses by U.S. and international research bodies have concluded that the lack of such knowledge and technologies is what is currently limiting the progress of translational and clinical applications of stem cells, and that their creation and implementation would be transformative to biomedicine. The NIH/NCATS Stem Cell Technology Facility (SCTF), the new phase of the NIH Center for Regenerative Medicine (NCRM) Common Fund Program, is ideally positioned to address the key knowledge gaps in the stem cell field. The mission of the Facility is to tackle the top scientific and technical problems that currently impede therapeutic use of induced pluripotent stem cells. The Facility will collaborate with researchers across various disciplines to validate the methods developed within for suitability in regenerative medicine applications. An overview of the facility will be presented, along with emerging small molecule screening technologies developed at NCATS as well as novel quantitative approaches (i.e. proteomics and phosphoproteomics), which should help to firmly establish human stem cells for drug discovery.



Pseudo-colored scanning electron micrograph of retinal pigment epithelium (RPE) monolayer derived from human iPS cells growing on a fibrous scaffold. Scaffold is colored blue, cell nuclei/cytoplasm brown, and RPE apical processes light green. *Vladimir Khristov, Nathan Hotaling, Kapil Bharti (NEI)*

Poster Abstracts

1 Astrocytes in Pathophysiology of GBA1-associated Parkinson Disease

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Parkinson disease (PD) is caused by the disruption of dopaminergic neurotransmission in the basal ganglia, associated with a severe reduction in the number of dopaminergic neurons in the substantia nigra and the formation of cytoplasmic inclusions called Lewy bodies. Currently, the role of glial cells such as astrocytes in Parkinson disease pathogenesis is unknown. Astrocytes provide critical support for neuronal function by processes such as providing antioxidant protection, clearing glutamate, developing and/or maintaining the blood-brain barrier, and releasing gliotransmitters and cytokines. Mutations in *GBA1*, the gene encoding glucocerebrosidase and mutated in the lysosomal disorder Gaucher disease, are the most common genetic risk factor for Parkinson disease. We generated astrocytes from iPSCs derived from fibroblasts of six patients with type 1 Gaucher disease, three with and three without parkinsonism, including two siblings discordant for Parkinson disease. The astrocytes exhibited decreased glucocerebrosidase activity and stored the glycolipid substrates glucosylceramide and glucosylsphingosine when compared to a healthy control. Moreover, astrocytes from patients with Gaucher disease and Parkinson disease had a decreased intracellular calcium response to ATP, an important molecule in glial-neuronal signaling. However, when conditioned medium from dopaminergic neurons was added to the iPSC-derived astrocytes, from patients with Gaucher and Parkinson disease showed increased inflammasome activation and Il-1β secretion. These results suggest that astrocytes may play an active role in driving neuroinflammation in *GBA1*-associated Parkinson disease.

2 High-Throughput Screening for Identifying Candidate Drugs to Prevent Proteotoxic Stress-Induced Retinal Pigment Epithelium (RPE) Cell Death

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Retinal pigment epithelium (RPE) degeneration is a likely cause of age-related macular degeneration (AMD).

RPE degeneration is associated with accumulated proteotoxic damage in the tissue. Using high-throughput screening (HTS) assays, we identified pharmacologically active compounds that can mitigate the proteotoxic stress on the RPE cells. These compounds provide potential drug candidates for treating AMD and other RPE-related degenerative diseases. For our HTS assays, we maintained iPS cell-derived RPE monolayer in 384-well plates, and optimized a known endoplasmic reticulum (ER) stressor to cause 50% RPE cell death in 48 hours. A library of pharmacologically active compounds (LOPAC) was tested in our assays at 9 μM and 46 μM concurrently with 10 μM of the ER stressor for a 48 hour-incubation. Cell viability was measured using CellTiter-Glo, a luminance based ATP-release assay. Among the 1280 LOPAC candidates tested in our primary assays, all of the compounds were toxic at 46 μM. 40 compounds were efficacious at preventing stress-induced cell death at 9 μM. Follow-up assays are currently being performed to screen for secondary hits. Confirmed secondary hits will be tested in animal models of RPE degeneration.

3 *Versatile Pluripotent Stem Cell Culture Platforms for Regenerative Medicine and Drug Discovery*

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Efficient generation of differentiated mature cells from human pluripotent stem cells (hPSCs) is a vital issue for stem-cell-based regenerative medicine and pharmaceutical applications. Conventionally employed colony-type culture methods often result in low cell yields and heterogeneous cell populations. To optimize current culture methods, we analyzed core signaling pathways that underlie epithelial-to-mesenchymal transitions (EMTs), cellular heterogeneity, and various hPSC growth patterns. We further developed new cell growth platforms based on non-colony type monolayer (NCM) on Matrigel, which were mediated by various Rho-associated kinase (ROCK) inhibitors. These ROCK inhibitors include Y-27632, thiazovivin, Y-39983 (ROCK I inhibitor), and phenylbenzodioxane (ROCK II inhibitor). Human pluripotent stem cells cultured under NCM conditions retained the pluripotent state and the capacity to differentiate toward the three germ layers and their derivatives. However, microarray analysis of mRNA gene expression indicated that a subset of genes that are related to mesoendodermal differentiation have been altered, suggesting that these altered gene expression might influence the efficiency of directed differentiation. We further modified our basic NCM protocols without the use of ROCK inhibitors. One of such NCM methods is to cultivate hPSCs on defined extracellular proteins such as the laminin isoform 521 (LN-521) in the presence or absence of E-cadherin. Modified NCM methods provided versatile platforms for genetic analysis of hPSCs and their differentiated cells. We found that NCM-based cell cultures are efficient for transfection of small oligonucleotide-based microRNAs and short-hairpin RNAs, DNA plasmids, and lentiviral particles. Moreover, we have used genetically modified cells for high throughput (HTP) assays in a 384-well format for drug discovery and directed differentiation. Thus, NCM-based methods overcome the major shortcomings of colony-type culture, particularly suitable for producing large amounts of homogeneous hPSCs for future clinical applications, drug discovery, and disease modeling.

4 *Using Induced Pluripotent Stem Cells to Study the Effects of Survival Motor Neuron Deficiency in Astrocytes*

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Spinal muscular atrophy (SMA) is an autosomal recessive neuromuscular disorder characterized by progressive,

often fatal muscle weakness, primarily attributed to the loss of motor neurons. SMA is the most common inherited cause of death in infants and young children. The incidence of SMA is 1-6000 to 1-10000 live births, and the carrier rate is 1-50 to 1-40. SMA is caused by the homozygous deletion or mutation of the survival motor neuron gene 1 (SMN1) leading to the loss of SMN protein. Humans have a homolog of SMN1, SMN2, which primarily encodes a highly unstable and rapidly degraded protein product, due to a nucleotide substitution that results in the exclusion of exon 7. Disease severity inversely correlates with SMN2 copy number, demonstrating the central importance of the SMN protein to the disease state.

Induced pluripotent stem cells (iPSc) have been reprogrammed from fibroblasts derived from two SMA patients and from a related carrier. Both patient cell lines have two copies of SMN2 and the total deletion of SMN1. SMN protein levels are depressed in both patient iPSc lines. These cells have been differentiated to motor neurons and can be used as a cell model of SMA. We are presently using these cell lines to assay motor neuron health when cultured with astrocytes, which have depleted SMN.

Astrocytes are the major support cell of the CNS. They are responsible for glutamate clearance and protection from excitotoxicity, for metabolic support of the neurons, for protection from injury and can aid or impair axonal regeneration. Astrocytes have been linked to increases in severity of other neurodegenerative disorders and in SMA. Astrocyte specific rescue of SMN protein levels has been shown to increase survival in SMA model mice. However, the mode through which astrocytes work to exacerbate SMA is unclear and what aspect of astrocyte biology is dysfunctional in SMA in unknown.

Preliminary work has indicated that in SMA astrocytes are deficient in supporting motor neurons and that this deficiency may be minimized by elevating important secreted factors. Primarily, SMN-deficient astrocytes have depressed MCP1 levels compared to control astrocytes and MCP1 has been linked to neuroprotection after excitotoxicity and acute ethanol toxicity. Preliminary work has shown that MCP1 can stimulate neurite outgrowth.

5 *Hair Follicle Associated Pluripotent (HAP) Stem Cells Differentiate to Beating Cardiac Muscle Cells*

*Masateru Yashiro[1], Sumiyuki Mii[1], Ryoichi Aki[1], Yuko Hamada[1], Nobuko Arakawa[1], Katsumasa Kawahara[2,3], **Robert M. Hoffman[4,5]**, and Yasuyuki Amoh[1]*

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We have previously demonstrated that nestin-expressing hair-follicle-associated (HAP) stem cells from the upper part of the hair follicle differentiate to neurons and other cell types (Proc. Natl. Acad. Sci. USA 100, 9958-9961, 2003; Proc. Natl. Acad. Sci. USA 102, 5530-5534, 2005; Proc. Natl. Acad. Sci. USA 102, 17734-17738, 2005; Cell Cycle 7, 1865-1869, 2008; Cell Cycle 10, 830-839, 2011) and form the hair follicle sensory nerve (J. Cell. Biochem. 114, 1674-1684, 2013). In the present study, we demonstrate that HAP stem cells differentiate to beating cardiac muscle cells. HAP stem cells can differentiated into cardiac muscle cells, as well as neurons, glial cells, keratinocytes, and smooth muscle cells in DMEM containing 10% FBS. The differentiation potential to cardiac muscle is greatest in the upper part of the follicle. Nestin-expressing hair spheres, formed from HAP stem cells from the upper part of vibrissa hair follicle, also differentiated to beating cardiac muscle cells. HAP stem cells have many advantages over iPS and ES cells in that they are highly accessible from every individual, do not form tumors, and have no ethical issues. Therefore, HAP stem cells have clinical potential for heart regeneration, as well as for nerve and spinal cord repair.

6 iPSC-Derived Neural Cells That Carry a Genetic Variant Associated With Serious Mental Illness Display Decreased TRANK1 Expression That is Rescued by Sodium Valproate

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Biological characterization of genetic variants identified in genome-wide association studies (GWAS) of neuropsychiatric disorders remains a substantial challenge, impeding translation of GWAS results into better methods of diagnosis and treatment. Here we used human induced pluripotent stem cells (hiPSC) and their neural derivatives to characterize a common variant associated by GWAS with both bipolar disorder (BD) and schizophrenia. We measured the relationship between genotype and gene expression and tested the impact of commonly used therapeutic agents *in vitro*. We also assessed expression of correlated genes and gene networks. Cells carrying the risk allele (G) of the SNP, rs9834970, located about 15 kb downstream of *TRANK1* on chromosome 3p22, displayed lower baseline expression of *TRANK1* mRNA that was not observed in postmortem brain tissue. Chronic treatment with therapeutic dosages of valproic acid (VPA) normalized *TRANK1* expression in hiPSC-derived neural progenitor carrying one or two copies of the risk allele, but had no significant impact in cells carrying only the other (A) allele. VPA treatment also reduced expression of histone deacetylase I (*HDAC1*) in neural progenitor cells, consistent with the drug's known inhibition of histone deacetylase activity. In contrast, shRNA knockdown of *TRANK1* increased *HDAC1* expression, suggesting that TRANK1 and VPA may have reciprocal effects on histone deacetylase activity. This study represents an efficient strategy to explore the findings from GWAS studies with human iPSCs and demonstrates how post-GWAS investigations can help identify causal disease variants and enhance our understanding of biological disease mechanisms.

7 Human iPSC-Derived Motor Neurons for Modeling Neurological Diseases

*Carrie Chavez, Chris McMahon, Ben Meline, Jea Liu, and **Eugenia Jones***

Cellular Dynamics International a FujiFilm company, Madison, WI

The aim of this study was to produce motor neurons from human induced pluripotent stem cells (iPSC) with sufficient purity for use in a multitude of downstream assays including electrophysiological recordings using a multi-electrode array (MEA) system. In particular we wanted to produce motor neurons that could be cultured in defined conditions, over long periods of time, without being hampered by outgrowth from proliferative cell types or the need to plate on primary glia. Using a 3D differentiation protocol, adapted from published methods, we were able to produce motor neurons from iPSC, at greater than 50% purity as measured by Isl 1/2 and Tuj1 positive staining. These cells can be stored frozen, thawed, and cultured in media without glia for extended periods, simplifying experimental design and data interpretation. Using iPSC lines from multiple donors we demonstrated the protocol is robust, that motor neuron production is independent of the donor iPSC line. We collected ICC, qPCR and MEA data to characterize the motor neuron cells we produced. Taken together these data show the characteristics and utilization of iPSC-derived motor neurons.

8 Human Induced Pluripotent Stem Cell-Derived Neurons as a Reproducible System for Drug Discovery

Richard Josephson, Roberta Harvey, Hiroko Yokoe and Jonathan M. Auerbach

MTI-GlobalStem, Gaithersburg, MD

Neurotoxicity studies and compound screening for drug candidates for neurological and neurodegenerative disorders rely on mature cultures of neurons and other neural cells. Currently, primary rodent neurons are the widely accepted norm in drug screening and toxicity assays. Now, human induced pluripotent stem cells (iPSC) promise to offer *in vitro* models of neurological diseases and improve the validity of drug screening data. One hurdle to the acceptance of iPSC-derived neurons has been demonstrating that these neurons can mature and function *in vitro* in a manner similar to their primary rodent counterparts. MTI-GlobalStem has developed a novel system for the differentiation, cryopreservation, and maturation of human iPSC-derived neural cells yielding reproducible cultures of functional human neurons. Within this system, the neuronal progenitor cells can differentiate into mature neuronal cultures with functional properties such as evoked calcium responses and possessing mature synaptic properties. We compared the morphological and functional maturation of primary and iPSC-derived neurons, and their potential to achieve the highly reproducible cultures required for pharmacological assays. Synaptic responses in iPSC-derived neurons were measured and compared to those of rat cortical neuronal cultures to estimate their degree of functional synaptic maturation and activity in response to various pharmacological agents. In summary, MTI-GlobalStem has developed cells and an improved cell culture system for increasing the longevity of rat and human neurons in culture, supporting their morphological and functional maturation, making for reproducible, reliable cultures, and using our novel transfection reagents, allowing for easy regulation of gene expression within this system.

9 mRNA for Cell Fate Manipulation

Miranda Yang[1], Marcy Comly[2], Alice E. Chen[1,3*], John Hanover[2], **James Kehler**[1,2*]

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Induced pluripotent stem cells (iPSCs) are becoming an invaluable tool for neurodegenerative disease modeling and drug discovery, but the prospect of applying iPSCs in cell therapy is still greatly limited by the risk of genomic alterations using viral methods for gene delivery during the reprogramming process. Recent advances have addressed these concerns and demonstrated the potential of messenger RNAs (mRNAs) to reprogram somatic cells to iPSCs. However, currently available mRNA-based reprogramming systems have been shown to have low success rate compared to other non-integrative methods, mainly due to transfection-related toxicity. Here, we demonstrate an mRNA/microRNA reprogramming system using a new lipid transfection reagent that exhibits lower toxicity with high efficiency, and reproducibility when generating iPSCs from neonatal and adult human fibroblasts. Subsequent delivery of mRNAs encoding neuronal transcription factors such as *Ngn1* or *Ngn2* can be used to induce iPSCs to adopt a neuronal fate. Alternatively, the delivery of *Ngn2* and *Brn3a* can be used to trans-differentiate patient fibroblasts into sensory neurons. By either approach, optimized delivery of mRNAs can be used to generate defined cell lineages from patient samples to accelerate neurodegenerative disease modeling and drug screening programs.

10 Developing iPS Cell Derived RPE Tissue for Clinical Application

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Purpose: Age-related macular degeneration (AMD) is a leading cause of vision loss in the United States. The disease is thought to originate by a malfunctioning retinal pigment epithelium (RPE), which leads to photoreceptor cell death and vision loss. Previous work suggests replacing diseased RPE with healthy autologous RPE sheet can provide vision rescue for AMD patients. Here we use tissue-engineering approach combined with induced pluripotent stem (iPS) cell technology to develop an autologous RPE sheet on an artificial biodegradable scaffold and provide a summary of preclinical studies.

Methods: iPS cells are differentiated into RPE using a directed-differentiation protocol that generates RPE in three phases: neuroectoderm/RPE progenitors, committed RPE, and immature RPE. RPE cells at the immature RPE stage are purified and seeded onto biodegradable electrospun bi-layered poly lacto-co-glycolic acid (PLGA) based scaffolds. PLGA fibers with different PLGA molecular weight are electrospun in different orientations to generate scaffolds with optimized biodegradability and tensile strength. RPE tissue is validated using electron microscopy, immunostaining, gene expression analysis, fluid transport assays, and electrophysiological analysis. RPE tissue is tested in pre-clinical animals for safety and efficacy.

Results: Electrospun scaffolds with randomly oriented and heat fused 400 nm fibers coated with extracellular matrix proteins provide an optimal environment for RPE maturation. Electrophysiological experiments show that RPE cells on PLGA develop transepithelial resistance of more than several hundred ohms/cm^2, suggesting tight electrical contacts between neighboring cells. Gene expression, immunostaining, and electron microscopy analysis show that iPS cell-derived RPE cells resemble native RPE in their molecular and structural properties. RPE cells on PLGA scaffolds also have the ability to phagocytose photoreceptor outer segments and show ability to transport water from apical to basal sides. **Conclusions:** This work provides a fully-authenticated iPS cell derived RPE tissue for transplantation in pre-clinical animal models. This tissue behaves similarly to native tissue in its molecular and functional properties. This work also provides a protocol to develop a clinical-grade RPE tissue for transplantation in AMD patients.

11 High-Throughput Screening to Identify Compounds that Increase Fragile X Mental Retardation Protein Expression in Neural Stem Cells Differentiated from Fragile X Syndrome Patient-Derived Induced Pluripotent Stem Cells

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Fragile X syndrome (FXS), the most common form of inherited cognitive disability, is caused by a deficiency of the fragile X mental retardation protein (FMRP). In most patients, the absence of FMRP is due to an aberrant transcriptional silencing of the *fragile X mental retardation 1* (*FMR1*) gene. There is no cure for FXS and the available treatments only provide symptomatic relief. Given that *FMR1* gene silencing in FXS patient cells can

be partially reversed by treatment with compounds that target repressive epigenetic marks, restoring FMRP expression may be one approach for the treatment of FXS. We have developed and optimized a homogeneous and highly sensitive time-resolved fluorescence resonance energy transfer (TR-FRET) assay for FMRP detection in a 1536-well plate format. Using neural stem cells differentiated from a FXS patient-derived induced Pluripotent Stem Cell (iPSC) line that does not express any FMRP, we have screened a collection of about 5000 known tool compounds and approved drugs with this FMRP assay. We have identified six compounds that modestly increase *FMR1* gene expression in FXS patient cells. While none of these compounds resulted in clinically relevant levels of *FMR1* mRNA, our data provide proof of principle that this assay in combination with FXS patient-derived neural stem cells can be used in a high-throughput format to identify better lead compounds for FXS drug development.

12 Modeling Late-Onset Retinal Degeneration with Human iPSCs: Insights into the Shared Pathogenesis of Macular Degeneration

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Purpose: Late-Onset Retinal Degeneration (L-ORD) is an autosomal dominant disorder caused by a S163R mutation in CTRP5. The disease is characterized by delayed dark adaptation, long anterior lens zonules, and drusen-like deposits in the sub-retinal pigment epithelium (RPE). The purpose of our study is to deduce the mechanism by which mutated CTRP5 causes retinal degeneration using RPE derived from induced pluripotent stem cells (iPSCs) specific to L-ORD patients and their healthy siblings.

Methods: iPSCs were generated from skin biopsies obtained from two affected and two unaffected siblings from a L-ORD family using Sendai virus based transfection of OCT3/4, SOX2, c-MYC, and KLF4 factors. iPSCs were characterized for expression of pluripotent markers by immunofluorescence and karyotyped for genetically stability. iPSCs were differentiated into RPE cells (iPSC-RPE) using a developmentally-guided differentiation protocol. The iPSC-RPE cells were authenticated by gene expression, immunofluorescence, TEM, and physiology.

Results: We successfully generated and authenticated iPSCs and iPSC-RPE from a L-ORD family. We confirmed that patient iPSCs retained the S163R mutation in CTRP5 and control iPSCs retained the wild-type sequence. TEM images of healthy sibling- and L-ORD patient- iPSC-RPE confirmed their epithelial morphology, presence of apical microvilli, melanosomes, and tight junctions. Preliminary findings indicate L-ORD Patient iPSC-RPE have impaired calcium regulation. This is consistent with the impaired adhesion of L-ORD RPE to Bruch's membrane reported in the literature. Further investigation is currently underway to determine the disease mechanism.

Conclusion: The retinal degeneration in L-ORD patients resembles age-related macular degeneration (AMD), a multi-factorial late-onset disease. However, unlike AMD, L-ORD is a monogenic disease caused by a single missense (S163R) mutation in the CTRP5 protein, making it a powerful tool to study more genetically complex diseases such as AMD. Elucidating the mechanism by which mutated CTRP5 causes L-ORD will aid in understanding disease mechanisms in AMD and other retinal degenerations.

13 Hibernation in a Dish?: Induced Pluripotent Stem Cells from the Thirteen-Lined Ground Squirrel

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Hibernation is an effective strategy adopted by some animals to survive the harsh winter conditions. During hibernation, thirteen-lined ground squirrels (*Ictidomys tridecemlineatus*) lower their core body temperature to 2-8°C. When awakened from deep torpor, they can regain normal body temperature and motility within several hours. However, such drastic changes are stressful to the animal. For example, in many species, the microtubules, a major cytoskeletal component that neurons rely on to transport vital signals and materials and form neural circuits, dissemble and hence sabotage neuronal activity when the temperature drops substantially below 37°C. To study cellular changes and protective mechanisms under hibernation-like conditions in ground squirrels, a versatile in vitro tool would be very useful. We describe here the first successful generation of ground squirrel induced pluripotent stem cell (iPSC) lines created to address this need. Pluripotency of the cell lines was verified by both in vitro differentiation and in vivo teratoma formation and karyotyping confirmed the lines had the appropriate chromosome number for this species. Primary neonatal cortical cultures of ground squirrel neurons and iPSC-derived ground squirrel neurons were cultured at 4-8°C for several days to test their resilience compared to human iPSC derived neurons. We found that both ground squirrel neuronal cultures were indeed resilient to the temperature drop, maintaining their neuronal processes for several days, while the microtubule processes of human iPSC-derived neurons collapsed overnight. Further analyses on ground squirrel and human iPSC-derived neuronal transcriptomes and microtubule dynamics may unveil novel neural protective mechanisms that can inspire effective strategies to treat human neuronal injuries and diseases.

14 Identification of Surface Proteome for Human Retinal Pigment Epithelium

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Purpose: The degeneration of the retinal pigment epithelium (RPE) plays a major role in photoreceptor cell death and vision loss in age-related macular degeneration (AMD). Induced pluripotent stem (iPS) cells derived RPE has provided hope for an autologous therapy against AMD. However, the potential presence of undifferentiated cells in transplantable tissues increases the risks of tumor or teratoma formation. Here we propose to identify RPE surface proteome to discover a canonical set of membrane biomarkers that can be used to validate and purify iPS cell derived RPE.

Methods: Cell surface capturing (CSC) technology was used to identify N-glycosylated proteins present specifically on the apical and/or basal surfaces of primary human fetal RPE monolayer. Bioinformatics analysis based on *in silico* gene expression, the number of hits in CSC technology, predicted protein transmembrane domains, and predicted subcellular localization was used to select a sub-set of proteins for further validation. Gene expression was used to determine relative expression of selected genes in iPS cells, iPS cell-derived RPE, fibroblasts, and primary RPE. Immunostaining was performed to confirm relative apical or basal localization of selected sub-set of proteins.

Results: 1,800 protein tags were identified using CSC technology. Approximately, 200 genes were selected based on the number of predicted transmembrane domains (more than one) and the number of times they were identified in the CSC technology (more than one). These 200 genes were analyzed using EST profiles and *in silico*

subcellular localization and a list of 61 genes was identified as preferentially localized on the membrane in adult cells. m-RNA expression analysis for these 61 genes in iPS cell-derived RPE, fibroblasts, hfRPE, and iPS cells identified 36 candidates that were preferentially expressed in iPS cell-derived RPE as compared to fibroblasts and iPS cells. Immunostaining on many of these proteins has validated the increased expression in the iPS cell-derived RPE and hfRPE.

Conclusions: We have successfully identified previously known and unknown surface markers on primary human fetal RPE cells. This specific set of proteins is being used as surface markers to validate iPS cell derived RPE and to generate pure population of RPE cells for transplantation in AMD patients. These results will lead to safer and likely more effective cell-based therapy for AMD.

15 *Manufacturing Induced Pluripotent Stem Cells from Whole Blood in a cGMP Environment: Developing a Human Cellular Therapy for Advanced Dry Age-Related Macular Degeneration*

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In Japan, a clinical trial of induced pluripotent stem (iPS) cell therapy has received regulatory approval, and is underway, for treating "wet" age-related macular degeneration. However, there is currently no effective treatment for advanced "dry" age-related macular degeneration. Consequently, we are developing a method for manufacturing, under cGMP, iPS cells to be used in a clinical protocol treating this condition. However, there are many complications to creating clinically useable iPS cells, including source material and manufacturing conditions. While iPS cells have been derived from many somatic tissues, whole blood has many advantages over other sources, especially for elderly patients. Blood can be drawn in relatively small amounts by nurses without surgical intervention, and circulating CD34+ hematopoietic cells have been shown to be amenable to ex-vivo expansion and reprogramming. There is considerable variation in the techniques for safely handling and processing circulating blood, each with its set of advantages and problems. Our ability to develop and compare clinical manufacturing methods is unique due to experience with routine cell and blood processing done under cGMP at the National Institutes of Health Clinical Center. In this poster, we compare cGMP-compatible methods of removing red blood cells (debulking) from whole blood, isolating CD34+ cells from total white cells, ex-vivo culture systems for expanding cell numbers, and banking/cryopreservation of the purified, expanded CD34+ cells. In each case, the final population of CD34+ cells is assayed for purity, identity, potency, and sterility. Potency is measured by the frequency of iPS colony generation per 10,000 cells. It is anticipated that most of these methods will show no clear advantage over the others, and that the systems most compatible with clinical manufacturing concerns can be safely chosen for further optimization and development.

16 *Clinical Grade Culture Medium for Expansion, Large-Scale Culture in 2D and 3D Suspension Culture of Human Pluripotent Stem Cells*

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The lack of robust methods for clinical expansion of human pluripotent stem (hPS) cells continues to hamper clinical use. Here we report the development of a culture system for expansion of hPS cells that is totally free of human- or animal-derived components and chemically defined—containing only raw materials of clinical quality with traceable production processes. hPS cells expanded in T-flasks as a monolayer (2D culture) maintain high

expression of pluripotent stem cell markers and lack expression of differentiation markers over 10 passages, display long-term self-renewal potential, and retain stem cells characteristics and the capability to differentiate into all three germ layers. Moreover, the culture system utilizes a single-cell based protocol that enables expansion from single hPS cells, making it amenable to genome engineering and selection applications. Generation of clinically relevant quantities of hPS cells, ranging from 10^9 or beyond, is essential for clinical use. We demonstrate that the culture system also supports large-scale, non-adherent expansion of hPS cells in suspension culture in a perfusion bioreactor over 3-5 passages with a 7-fold increase ratio between passages. Thus, using 3D suspension cultures, hPS cells can be expanded > 16,000-fold within 5 passages over 18 days.

In summary, our study suggests that scale up of hPS cells can be performed under GMP conditions. The culture system supports the expansion of hPS cells both in 2D as well as in 3D, non-adherent suspension culture format. Critically, the expansion potential of this culture system has the capacity needed for clinically relevant quantities of hPS cells, even when starting from a single hPS cell.

17 iPSC and Neuronal Derivatives from a Patient with Bipolar Disorder and 574-kb Duplication on Chromosome 16p11.2

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Rare copy number variants (CNVs) have been shown to be associated with risk for diverse neurodevelopmental and neuropsychiatric disorders. Of these, the CNV on 16p11.2 that spans ~600 kb, contains deletions in autism spectrum disorder (ASD) and intellectual disability (ID), and duplications in ASD, bipolar disorder (BD), ID and schizophrenia (SCZ). We identified a BD patient with 16p11.2 duplication through SNP array genotyping. We also identified an unaffected sister without duplication in the same of affected Amish families. To investigate cellular phenotypes that may be associated with this CNV, we reprogrammed both the patient's and her unaffected sibling's fibroblasts. A total of eight induced pluripotent stem cell (iPSC) clones were isolated from each parental fibroblast line and all clones showed evidence of pluripotency through immunocytochemistry and fluorescence-activated cell sorting (FACS). Spectral karyotyping revealed a normal XX karyotype for three selected clones from each series. To examine distinct phenotypes in neuronal derivatives, iPSC clones are differentiated into neural stem cells/neural progenitor cells (NSC/NPC), neurons and astrocytes. Neuroprotection by mood stabilizers in the presence of stressors in these cells is also being evaluated. Gene expression profiles for each iPSC derivative as well as cellular and functional phenotypes, under various conditions, could reveal the molecular and biological impact of 16p11.2 duplication and may provide clues to the basis of its role in BD susceptibility.

18 Cell Type and Species Specific Toxicity Screen of Human Neural Stem Cells and Rat Cortical Neurons

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Human primary neural tissue is a vital component for the quick and simple determination of chemical compound neurotoxicity in vitro. In particular, such tissue would be ideal for high-throughput screens that can be used to identify novel neurotoxic or neurotherapeutic compounds. We have previously established a high-throughput screening platform using human induced pluripotent stem cell (iPSC)-derived neural stem cells (NSCs) and neurons. In this study, we conducted a 2000 compound screen with human NSCs and rat cortical neuronal cells to

identify compounds that are selectively toxic to each group. Approximately 100 of the tested compounds showed specific toxicity to human NSCs at a screening concentration of 10 micromolar. A confirmatory and secondary screen of a small subset of compounds from the primary screen on human iPSCs, NSC-derived neurons, and fetal astrocytes validated the results from more than 80% of these compounds, with some showing cell specific toxicity. Amongst those toxic compounds were several cardiac glycosides, all of which were selectively toxic to the human cells. As the screen was able to reliably identify neurotoxicants, many with species and cell-type specificity, this study demonstrates the feasibility of this NSC-driven platform for higher-throughput neurotoxicity screens.

19 *Primary Cilia Regulates the Polarization and Maturation of hiPSC-RPE*

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Retinal pigment epithelium (RPE), a ciliated monolayer of cells situated adjacent to retinal photoreceptors, is critical for maintaining the health and integrity of photoreceptors. Ciliopathies are a class of disorders that affect cilia formation or functioning and lead to photoreceptor degeneration. Similar to photoreceptors, RPE also contain a primary cilium during development. But the role of primary cilium in human RPE development or ciliopathies remains largely unknown. The goal of this study is to explore the function and underlying signaling pathway of primary cilium in RPE development and maturation using human induced pluripotent stem cell derived RPE (iPSC-RPE). Primary cilia in iPSC-RPE were manipulated using cilium inducers, blockers or specific protease inhibitors. Electrophysiology, immunocytochemistry, scanning and transmission electron microscopy, gene expression, and phagocytosis were used to determine the polarization and maturity of iPSC-RPE monolayer. Our results showed that progressive development of primary cilia in human iPSC-RPE coincides with the formation of RPE tight junctions and epithelial cell morphology. Experimentally enhanced activation of primary cilia led to extensive apical processes, improved pigmentation, increased expression of RPE signature genes, increased phagocytic capability, and significantly enhanced electrical responses that mimic native RPE, demonstrating improved RPE maturity and function. Electrophysiological recordings with pharmacological inhibitors of PKC-δ, in combination with the immunostaining for the active phosphorylated form of PKC-δ and its downstream target revealed that primary cilia-induced polarization and maturation of iPSC-RPE cells was regulated through the activation of PKC-δ pathway. These results indicate that the development of primary cilium in iPSC-RPE has a direct and dramatic impact on RPE monolayer polarization and maturation. It suggests a mechanism to mature RPE or other epithelial cell types derived from human iPSCs, and also provides insight into retinal degeneration caused by ciliopathies.

20 *Use of Induced Neural Stem Cells Derived from Human Hematopoietic Progenitor Cells for an In Vitro Autologous Model of Neuroinflammatory Diseases*

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Many undiagnosed disorders with neurological symptoms involve neuroinflammation. It is a challenge to determine whether the detrimental neuroinflammation is a result of the defect in neural cells or the malfunction of inflammatory cells. The main obstacle in such research is obtaining autologous inflammatory cells and neural cells from patients to reconstitute disorders in vitro. Recent advances in the field of cell transformation provide unique opportunities for in vitro human disorder modeling, such as using induced pluripotent stem cells (iPSC)-derived neurons for the study of neurological disorders. Based on our published method of directly generating

neural stem cells (iNSC) from human peripheral CD34 cells, we aimed to develop a convenient method to separate inflammatory cells and generate iPSC and iNSC from 10 ml of peripheral blood samples for constituting autologous neuroinflammation models. We optimized conditions of PBMC separation and CD34 cells purification, and further generated iNSC and iPSC simultaneously from CD34 cells transfected with Yamanaka factors with the help of selective media. The neural stem cells could be derived within two weeks after transfection and iPSC appeared in three weeks, as confirmed by immunostaining for cell specific markers. Meanwhile, we collected PBMCs from the same blood sample for co-culturing with the derived neural cells, thus creating an autologous model of neuroinflammation from as low as 10 ml of human blood. This method has been used to generate iNSC/iPSCs for a variety of neurological disorders and helped decipher the roles of inflammatory cells and neural cells played in the neuroinflammation.

21 CGG-Repeat Instability in Fragile X Syndrome Patient-Derived Stem Cells

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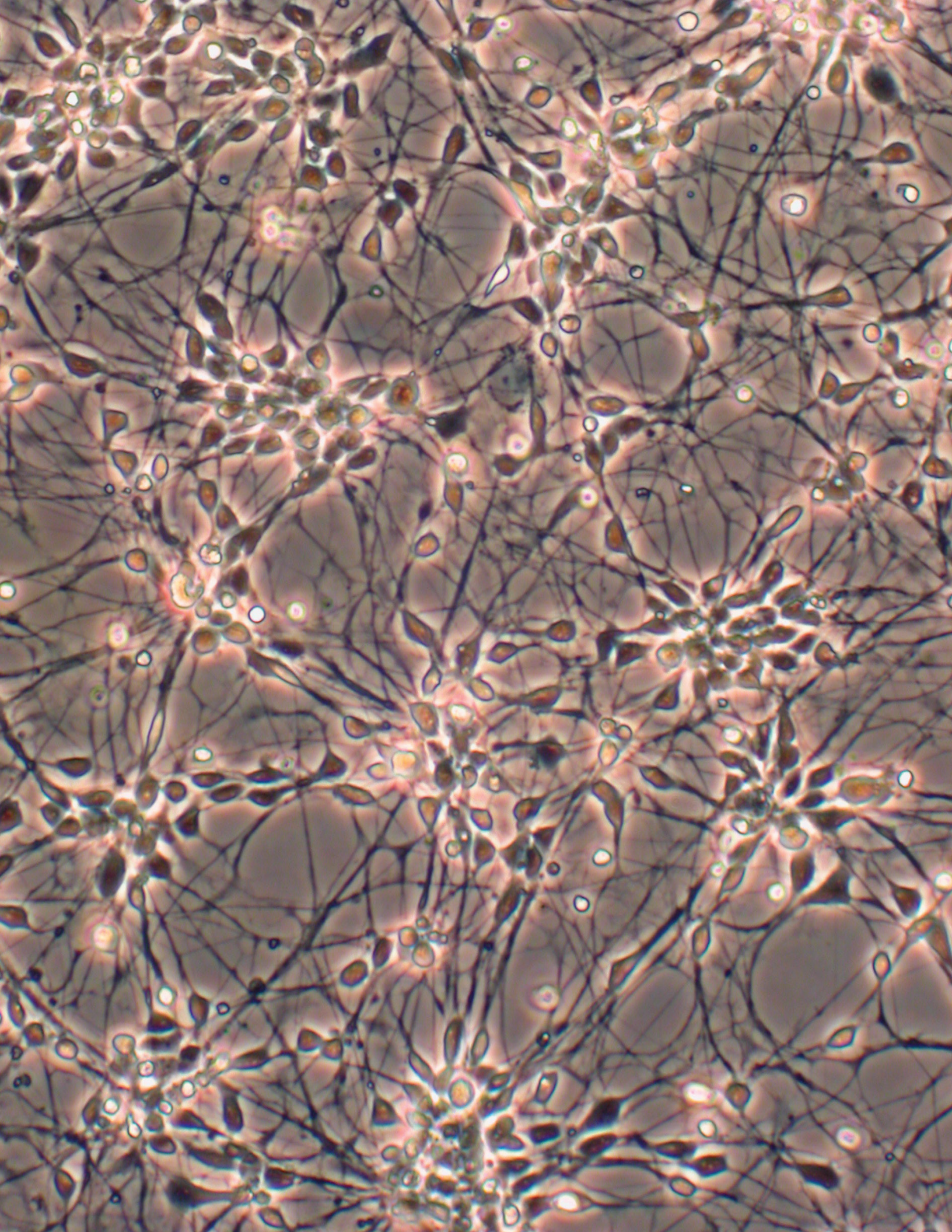
Fragile X syndrome (FXS) is caused by expansion of a CGG-repeat tract in the 5' UTR of the fragile X mental retardation (*FMR1*) gene that results in repeat-mediated *FMR1* gene silencing. The lack of a good tissue culture model has hampered efforts to understand the expansion mechanism as well as the mechanism of gene silencing. Work in affected humans and in mouse models has suggested that expansion may occur during oogenesis or the early stages of embryogenesis and that silencing occurs during embryonic development. In other diseases caused by repeat expansion, continuous expansion has been observed in embryonic stem cells (ESCs) or induced pluripotent stem cells (iPSCs) derived from affected individuals and in both mouse and humans expansions are seen in brain.

The purpose of this study was to establish a human cell culture model for studying repeat expansion. The stability of the repeat was examined in patient-derived iPSCs and in neural progenitors and neurons derived from these iPSCs as well as in individual clones isolated from a FXS hESC line. No expansions were seen on propagation of the iPSCs, however, a small expansion was observed upon their differentiation into neuronal cells. No further expansion was seen when the neurons were grown for nine weeks. Expansions were also not seen in various clones derived from FXS hESCs on continuous culture. This may indicate that most expansions occur prezygotically, consistent with observations from our FX mouse model. In contrast, contractions were seen in both ESCs and iPSCs. Similar contractions in the early human embryo may contribute to the frequent mosaicism for different repeat lengths that are seen in humans with FXS. These cell lines may thus be useful for understanding the contraction mechanism. In the course of propagation of hESCs clones that were mosaic for methylated and unmethylated alleles, a selective growth advantage was observed for the methylated allele. This may have important implications not only for our understanding of the silencing process but also for our understanding of the disease pathology seen in individuals with large, unsilenced alleles.



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