NRC newsletter

http://med.uth.edu/nrc | News & Featured Research of the Neuroscience Research Center | volume 21, number 1, Winter 2015

Gene expression and regulation in the central nervous system

By Jiaqian Wu, Ph.D.

Wu

Abstract: The central nervous system (CNS) is very complex and is comprised of diverse classes of cells that differ in their developmental processes, metabolism, signaling, and function. The complexity of RNAs expressed in neural tissues is also very high, making a comprehensive analysis of gene expression as well as RNA isoforms challenging. Such information is crucial for understanding mechanisms of the molecular basis of CNS function, disease and regeneration. The goal of my lab is to understand gene transcription and regulation in CNS using interdisciplinary approaches.

The complexity of gene expression in the CNS exists at several levels, including the mechanisms regulating gene structure as well as how gene variants are generated. This complexity underlies the regulation of CNS function; both under normal and pathological conditions. My laboratory uses systems-based approaches involving cell biology, genomics, proteomics, and bioinformatics in conjunction with functional assays to unravel gene transcription and regulatory mechanisms governing neural differentiation.

The systematic dissection of an organ to its primary cell type components for the purpose of establishing a transcriptome database is an essential step towards understanding a complex tissue like the brain. Together with neurons, glia (astrocytes, oligodendrocytes, microglia) and vascular cells (endothelial cells and pericytes) are crucial for nervous system function. Each of these major cell types express a distinct repertoire of genes known as a transcriptome. Studies that focus on the response of these transcriptomes to a range of stimuli (developmental, pathological, etc.) can provide fundamental insights into the development and function of the CNS. Understanding CNS function (as well as dysfunction)

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Treating spinal cord injury using human iPSC derived neural progenitor populations

By Ying Liu, M.D., Ph.D.

Liu

My research makes use of human induced pluripotent stem cells (iPSCs) and direct lineage reprogramming technologies for disease modeling, drug discovery, and neural regeneration and repair. Currently, I am developing novel methodologies to identify, purify and deliver iPSC-derived stem or progenitor cells, which are both safe and effective therapeutics, for the treatment of spinal cord injury and stroke. This work will help provide a better understanding of stem cell growth and differentiation, and elucidate mechanisms of central nervous system (CNS) development, injury and inflammation, and pathogenesis of CNS disease.

Generation of iPSCs and neural populations from cells obtained from patients' urine and application in a clinically-relevant rodent model of spinal cord injury (SCI)

Clinically safe neural stem cells and/or neural progenitor cells are long-sought resources for the treatment of CNS trauma and injury. Due to the limited availability and ethical issues of fetal derived neural tissues, a rising star in the stem cell field is the iPSCs. Human iPSCs, which are reprogrammed from somatic cells, have the potential to circumvent some of these problems by offering unlimited self-renewal capacity, and the potential for producing/manufacturing purified and homogenous neural progeny populations in large quantities.

Since the discovery of iPSCs in 2006, reprogramming protocols have quickly evolved to meet an ever growing array of

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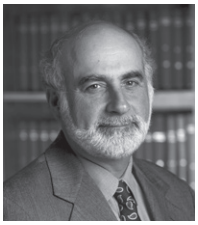
UTHealth | Neuroscience Research Center
The University of Texas Health Science Center at Houston

Director's Column

From the Director, John H. Byrne, Ph.D.



The start of a new calendar year brings forth great expectations for the year ahead. New developments over the past two years have laid the framework for expanding growth of the UTHealth Neuroscience Research Center and new collaborations. In 2013, when President Obama announced the Brain Research through Advancing Innovative Neurotechnologies (BRAIN) Initiative, the necessity and urgency of advancements in neuroscience was brought to the national platform. This announcement has driven the creation of multiple institutes and programs across the United States with one goal: to accelerate the development and application of new technologies that will provide insight into nervous system function in health and disease. Never has there been such a focused and coordinated effort to further the understanding of the brain.

The University of Texas System has seized this grand challenge and created a virtual institute to advance neuroscience and neurotechnology research. This new institute, the UT System Neuroscience and Neurotechnology Institute, will help Texas neuroscientists compete for federal and private grants by awarding seed funding for researchers at UT System components and their collaborators at other Texas institutions. It will also help to recruit new faculty, and fund the purchase and creation of innovative technologies. This organized effort is aimed to give Texas neuroscientists an edge when competing for federal and private grants in an increasingly difficult funding environment.

At a more local level, and based on this Initiative, the UTHealth Neuroscience Research Center has paired with the UTHealth Center for Clinical and Translational Sciences (CCTS) to provide a funding opportunity, the UTHealth BRAIN Initiative. This program will seed eight pilot projects in the critical areas outlined in the federal BRAIN Initiative to further our understanding of the relationship between cells, circuits and behavior. Collaboration amongst UTHealth institutions as well as other local institutions was highly encouraged in the application announcement. The recipients of these awards as well as information about their proposal are listed on our website.

The Center's annual fall programs were very well received. Dr. Ponnada Narayana, Professor of Radiology and Director of MR Research at UTHealth, directed our Neurobiology of Disease course. The topic this year was Central Nervous System Imaging in Health and Disease and educated graduate students, medical students and postdoctoral fellows on advancements in neuroimaging of normal brain development, psychiatric disorders, epilepsy, and multiple sclerosis. A highlight of the course was a special guest lecture by Dr. Peter Fox, an international authority on neuroimaging and handling large image data. Two other annual fall events include a reception at the meeting of the Society for Neuroscience, held this year in Washington, DC, and a local neuroscience poster session. In DC, our largest group of current and former UTHealth neuroscientists met up for some authentic Texas barbeque to discuss their research and future collaborations. In Houston, the 21st annual Neuroscience Poster Session included neuroscientists from the UTHealth NRC, Baylor College of Medicine Department of Neuroscience, and Rice University's Departments of Psychology, and Electrical and Computer Engineering. Both events were filled with lively discussions about the current status and future growth within the field.

We have many wonderful programs coming up this spring. Please be sure to check our website, social media pages, and Neurofax calendar to stay up to date on our events, as well as other local neuroscience events in the Texas Medical Center.

As a final note, I would like to personally thank Dr. Raymond Grill for his years of service as the Editor of the NRC newsletter. He was very committed to the success of the newsletter and many other NRC events. We wish him the best of luck at the University of Mississippi Medical Center, where he will continue to build a strong research program. Dr. Anne Hart, the Program Manager for the NRC, will take his place as Editor.

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requires an understanding of how distinct cell classes interact in a dynamic environment.

Currently most publicly available gene expression data were generated from tissue homogenates. Gene expression occurring in rare cell types may go undetected, as they only comprise a small portion of the total tissue RNA. Moreover, the majority of previous transcriptome studies were performed using microarrays, a hybridization based technology, where transcript abundance is indirectly deduced from intensities of fluorescence signals. Although the use of microarray technology has provided valuable insights into the neurological disorders, microarray technology suffers from limitations in resolution, dynamic range and accuracy. Another method RNA Sequencing (RNA-Seq) profiles the transcriptome by deep sequencing isolated RNAs, such that transcript abundance is directly proportional to the number of sequencing reads that map to a specific transcript. We, and others, have found that RNA-Seq has several advantages over microarrays. First, sequencing technology is much more sensitive and quantitative than microarrays. Second, RNA-Seq provides a larger dynamic range of detection of transcripts (> 9,000-fold) compared to standard arrays. Third, sequencing data are more specific and have less background. Furthermore, sequencing experiments do not depend on the limited probes of tiled microarrays and can therefore be used to interrogate any location in the genome. Finally, sequencing is not limited by array hybridization chemistry, such as melting temperature, cross-hybridization, and secondary structure concerns. Importantly, RNA-Seq also enables splicing isoform identification and expression level estimation by providing splice junction reads.

To better understand the functions and interactions of the cell types in the brain, we acutely purified representative populations of neurons, astrocytes, oligodendrocyte precursor cells, newly formed oligodendrocytes, myelinating oligodendrocytes, microglia, endothelial cells, and pericytes from mouse cerebral cortex (Figure) (Zhang et al. J Neurosci., 34: 11929, 2014). We generated a high resolution transcriptome database of > 22,000 genes for these eight cell types by RNA sequencing and used a sensitive algorithm to detect alternative splicing events in each cell type. Bioinformatic analyses identified thousands of new cell type-enriched genes and splicing isoforms that provide novel markers for cell identification, tools for genetic manipulation, and insights into the biology of the brain. Previously undetected cell type specific transcription factors, secreted ligands and membrane receptors, ion channels, cell adhesion molecules, and enzymes were also found. Perhaps most interestingly, our data can be used to better understand differences in glial and neuronal function. For example, our data provides clues as to how astrocytes and neurons differ

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***The NRC* would like to express thanks to Dr. Raymond Grill for his leadership through his role as Editor of the NRC Newsletter over the past four years.**

Dr. Grill has consistently contributed to the advancement of the mission of the NRC throughout his career at UTHealth. In addition, he has been a dominant figure in the evolution of the annual Neuroscience Poster Session through his participation as mentor and judge. Dr. Grill's contribution to NRC sponsored events during Brain Awareness Week makes him a valued member of the NRC community. We wish him all the best in his new role as Associate Professor at the University of Mississippi Medical Center.

Congratulations to NRC Members:

Kit Sing Au, Ph.D., Assistant Professor of Pediatrics, and Hope Northrup, M.D., Professor of Pediatrics and Director of the Division of Medical Genetics, received four research grants together: 1) "Creating a myelomeningocele exome variant map" from NIH/NICHD; 2) "Early biomarkers of autism spectrum disorders in infants with tuberous sclerosis complex" from NIH/NINDS/NICHD; 3) "Identification of potential EEG biomarkers and antiepileptogenic treatment strategies for epilepsy in TSC" from NIH/NINDS; and 4) "Developmental synaptopathies associated with TSC, PTEN and SHANK3 mutations" from NIH.

Raymond Cho, M.D., Associate Professor of Psychiatry and Behavioral Sciences and Director of the Psychosis Recovery and Research Center, received a grant from NIMH to investigate the interactions between gamma oscillations and lower frequency rhythms and how they are disturbed in early psychosis, using sensory and cognitive paradigms, MEG and computational modeling approaches.

Raymond J. Grill, Ph.D., Associate Professor of Integrative Biology and Pharmacology, and Scott D. Olson, Ph.D., Assistant Professor of Pediatric Surgery, received a Bentsen Stroke grant titled, "Novel application of adult, human mesenchymal stem cells to reduce inflammation and treat neuropathic pain in a clinically-relevant rat model of chronic spinal cord injury."

Rodrigo Hasbun, M.D., M.P.H., Associate Professor of Internal Medicine, received an NIH grant to evaluate the long term neurological, neurocognitive and renal outcomes of patients with West Nile virus infection. He also received another NIH grant to collect and distribute critical clinical data and tissue specimens from longitudinally-tracked HIV-infected individuals at high risk for HIV-associated neurocognitive disorders.

Ruth Heidelberger, M.D., Ph.D., Professor of Neurobiology and Anatomy, was the Convocation speaker for Stony Brook University, Department of Chemistry in 2014, and was recently elected to the Council of the Biophysical Society. She also received an NIH competitive renewal of her R01 to determine the roles of Ca2+ and downstream signaling molecules in the regulation of neurotransmitter release in the mammalian retina.

Georgene Hergenroeder, R.N., M.H.A., CCRC, Assistant Professor of Neurosurgery received a grant from the Texas Department of State Health Services to facilitate transition of youth with chronic disability/long-term health care needs to adult care, and to provide education in this area. She also received a grant from Mission Connect and the TIRR Foundation to evaluate plasma vitronectin levels in neuropathic pain in spinal cord injury.

Cameron Jeter, Ph.D., Assistant Professor of Diagnostic and Biomedical Sciences, UTHealth School of Dentistry, received a grant from the Albert and Ethel Herzstein Charitable Foundation Geriatric Studies for Junior Faculty, UTHealth Consortium on Aging, to examine oral health in Parkinson's and Huntington's Diseases. She also received the Dean's Excellence Award, Scholarship of Teaching, UTHealth School of Dentistry.

Stephen Mills, Ph.D., John McGovern Distinguished Professor of Ophthalmology and Visual Science, received a research grant to study the blue-green pathways in the mammalian retina from the NIH National Eye Institute.

Shin Nagayama, Ph.D., Assistant Professor of Neurobiology and Anatomy, received a research grant from the NIH to categorize the multiple types of neurons in the olfactory bulb and elucidate the functional contributions of the neuron to the odor information process.

Ponnada Narayana, Ph.D., Professor of Diagnostic and Interventional Imaging, recently received three research grants: 1) "Lesion activity and atrophy in multiple sclerosis: Analysis of multi-center MRI" from NIH/NINDS; 2) "Effects of teriflunomide on regional cortical and deep gray matter atrophy: Analysis of the EFC6049 MRI data" from Genzyme Corporation; and 3) "Effect of repeated Gd administration: Controlled studies in rodents" from the National Multiple Sclerosis Society.

Flavia M. Nelson, M.D., Associate Professor of Neurology, received a K-23 grant from the NIH/NINDS for the grant titled "Detection of multiple sclerosis related cognitive impairment: In search of MRI surrogate markers."

Scott D. Olson, Ph.D., Assistant Professor of Pediatric Surgery, received an investigator-proposed grant funded by Genzyme Corporation to test their MS drug, teriflunomide or Aubagio, to treat severe and mild-moderate TBI in a rat model.

John Redell, Ph.D., Assistant Professor of Neurobiology and Anatomy, received a grant funded by the TIRR Foundation and Mission Connect for the study titled "Improving learning and memory in brain injured animals."

Dr. Xiaoqian Naomi Fang, from the laboratory of **Teresa Santiago-Sim, Ph.D.,** Assistant Professor of Neurosurgery, received a 2014 Fall Postdoctoral Association Travel Award.

Anne Sereno, Ph.D., Professor of Neurobiology and Anatomy, received a grant funded by the TIRR Foundation and Mission Connect for the study titled, "Development of behavioral biomarker of acute concussion for predictors of post-concussion symptoms."

Laura Smith Callahan, Ph.D., Assistant Professor at the Institute of Molecular Medicine, received a Bentsen Stroke Center grant for a study titled, "Advanced artificial extracellular matrix for treatment of chronic stroke." She also received a grant from Mission Connect, a program of TIRR Foundation, for the study titled, "Optimization of tissue engineering matrices for SCI treatment."

Claudio Soto, Ph.D., Professor of Neurology, received a grant from The Michael J. Fox Foundation for Parkinson's disease for a study on the detection of alpha-synuclein oligomers for the diagnosis of Parkinson's disease. He also received a grant from the NIH/NIAID to examine mechanisms of transmissibility in prion diseases with particular emphasis on the study of human prion diseases.

Nitin Tandon, M.D., Associate Professor of Neurosurgery, recently received four research grants: 1) "Mapping human memory with Electrocorticography and Chronometric Stimulation" from the University of California and NIH; 2) "Collaborative research: Exploring sparsity and spectral-temporal decomposition in real-time network modulation for intractable epilepsy" from the National Science Foundation; 3) "Spectro-spatial topography of human cerebral cortex" from Rice University and NIH; and 4) "How inhibitory control modifies stimulus value and motivation" from the University of California San Diego - NIH / NIDA.

Pamela L. Wenzel, Ph.D., Assistant Professor of Pediatric Surgery, received a grant funded by the TIRR Foundation and Mission Connect for the study titled "Preconditioning of mesenchymal stromal cells as cellular therapeutics for traumatic brain injury."

Jia Qian Wu, Ph.D., Assistant Professor of Neurosurgery, received a grant through Mission Connect and the TIRR Foundation to find therapeutic targets for chronic SCI using rat contusive injury model.

Nuray Yozbatiran, Ph.D., Assistant Professor of Physical Medicine and Rehabilitation, received a grant funded by the TIRR Foundation and Mission Connect for the study titled "Effects of combined cerebral and spinal direct current stimulation on upper limb recovery in incomplete spinal cord injury."

21st Annual Neuroscience Poster Session

Drs. Jack Byrne (UTHealth), Dora Angelaki (BCM), Simon Fischer-Baum (Rice), and Behnaam Aazhang (Rice).

Group shot of faculty judges from all three institutions

Saturday, December 6, 2014
UTHealth Cooley
University Life Center

The UTHealth NRC was pleased to organize and co-host the 21st Annual Neuroscience Poster Session with Baylor College of Medicine (BCM) Department of Neuroscience, and Rice University Departments of Psychology and Electrical and Computer Engineering. Sixty-three posters were presented by faculty, research scientists, graduate and medical students from all three institutions. We are grateful for the generosity of thirty-two faculty judges who volunteered their Saturday morning, and local establishments that donated some great door prizes.

Group shot of presenters from all three institutions

Congratulations to all of the winners from the 21st Annual Neuroscience Poster Session:

Graduate Student Awards:

UTHealth Graduate School of Biomedical Sciences 1st Place: The Dee S. and Patricia Osborne Endowed Scholarship in the Neurosciences: Curtis Neveu

Rice University Gertrude Maurin Cognitive Neuroscience Research Award: Yingying Tan

Rice University Award from the Department of Electrical and Computer Engineering: Rakesh Malladi

Baylor College of Medicine Award from the Department of Neuroscience: Kuchuan Chen

2nd Place Prizes:
Qiuhai Yue (Rice Psychology) and Adhira Sunkara (BCM)

3rd Place Prizes:
Jenny Sun (BCM) and Yu-Mei Huang (BCM)

Postdoctoral Fellow Awards
1st Place: Richard Dewell (BCM)

2nd Place: Alexis Bavencoffe (UTHealth)

3rd Place: Aram Giahi Saravani (BCM)

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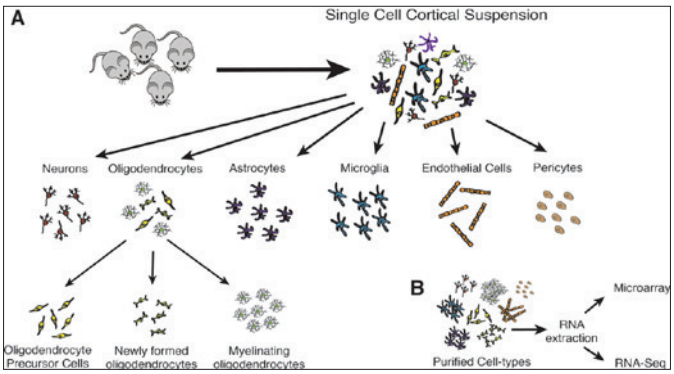

Purification of neurons, glia, and vascular cells from mouse cerebral cortex using a combination of immunopanning and FACS procedures

in their ability to dynamically regulate glycolytic flux and lactate generation attributable to unique splicing of Pkm2, the gene encoding the glycolytic enzyme pyruvate kinase. Accumulating evidence has demonstrated that glia are involved in a variety of neurological diseases, including schizophrenia, autism spectrum disorders, epilepsy, Alzheimer's disease, Parkinson's disease, multiple sclerosis, amyotrophic lateral sclerosis, and stroke. As the research community continues to look beyond neurons in order to understand the pathogenesis of neurologic dysfunction, the comprehensive transcriptome dataset of the brain presented here will be a valuable resource.

Alternative splicing of specific genes also plays an important role in the development and function of the nervous system. Before systematic methods for the analysis of alternative splicing became available, characterizing alternative splicing of specific molecules and their role in the nervous system was cumbersome and time-consuming. Previous EST (Expressed Sequence Tags) mapping studies led to the conclusion that the brain has the highest number of alternative splicing events compared to other organs, with different brain regions exhibiting complex patterns of alternative splicing. However, the cellular source of this complexity is not known because analyses were carried out on brain tissues comprised of mixed cell types. In our study, we generated a high quality splicing dataset for every gene in the major cell classes of the brain and identified interesting splicing patterns of considerable biological significance. We found that alternative splicing occurs as frequently in glia and vascular cells as it does in neurons. In contrast to the overall similarity in the frequency of alternative splicing, each cell type contains its own specific repertoire of thousands of alternatively spliced RNAs. These observations suggest that the distinct functions of each cell type may result as much from the presence of specific protein isoforms generated by alternative splicing as from global differences in the levels of gene expression. In addition, previous studies have shown that disruption of alternative splicing can lead to neurological diseases in human and animal models, and our splicing dataset provides a resource for understanding the cell type-specific contributions to these disorders.

The complete datasets of brain cell types are deposited in a database displayed using an interactive web browser (http://jiaqianwulab.org/resource.htm) that provides readily accessible platforms for analyzing and comparing transcription and alternative splicing profiles for various cell classes in the brain. Hopefully, this resource will help to advance the understanding of the development and function of nerve cells and glia.

In addition to studying purified cell types from animals, the analysis of the gene expression during stem cell neural differentiation is another powerful approach to provide insights into the mechanisms and pathways involved in early cell fate specification. An example is the acquisition of neurogenic potential and the transition to gliogenic potential, which may ultimately be extremely useful for pharmacological screening leading towards the development of neurodegenerative disease and trauma-based therapies. In order to examine the fundamental mechanisms governing neural differentiation, we analyzed the transcriptome changes that occur during the differentiation of human embryonic stem cells (hESCs) into the neural lineage (Wu et al. Proc Natl Acad Sci USA, 107: 5254, 2010). Undifferentiated hESCs, as well as cells at three stages of early neural differentiation, N1 (early initiation), N2 (neural progenitor), and N3 (early glial-like), were analyzed using a combination of RNA sequencing technologies. The results revealed enormous complexity in gene transcription and splicing dynamics during neural cell differentiation. We found previously unannotated transcripts and spliced isoforms specific for each stage of differentiation. Interestingly, splicing isoform diversity is highest in undifferentiated hESCs and decreases upon differentiation, a phenomenon we call "isoform specialization." During neural differentiation, we observed differential expression of many types of genes including those involved in key signaling pathways, and a large number of extracellular receptors exhibiting stage-specific regulation. These results provide valuable information for the mechanisms underlying neural differentiation of hESCs. In addition, together with Dr. Ying Liu's group at UTHealth, we are currently studying hiPSC-derived neural stem cells (NSCs) and neuronal restricted progenitors (NRPs) with the goal to increase the neuronal differentiation efficiency of NSCs, which is highly valuable for developing effective treatment to repair a damaged nervous system.

Characterizing gene expression and regulation in the central nervous system can ultimately lead to solutions and therapeutic strategies for neurological disorders. For example, we have characterized the temporal gene expression changes in global gene expression after contusive spinal cord injury (SCI) in mouse and rat injury models, collaborating with Dr. QIlin Cao's group at UTHealth (Chen et al. PLoS One, (8):e72567, 2013). SCI is a devastating neurotrauma without effective treatment. To generate a comprehensive view of the mechanisms involved in SCI pathology, we sequenced mouse and rat spinal cord samples from acute and chronic phases (2 day, 7 day, 1 month, 3 month, 6 month and 1.2 year after injury) and systematically characterized the transcriptomes with the

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goal of identifying pathways and genes critical in SCI pathology. We developed a systems-based analysis framework in order to identify key determinants in the global gene networks. Some candidate genes that we identified have been shown by other groups to play important roles in SCI, which demonstrates the validity of our approach. We also identified many genes whose functions in SCI have not been well studied and can be further investigated by future experiments. In addition, we have incorporated pharmacogenomic information into our analyses. Among the genes identified, the ones with existing drug information can be readily tested in SCI animal models. Therefore, in this study we have shown an example of how global gene profiling can be translated to identifying genes of interest for functional tests and generating new hypotheses. We are in the process of testing the potential therapeutic targets and drugs in animal models of SCI.

Overall, our interdisciplinary research on gene transcription and regulation in the nervous system under normal and diseased conditions will advance the understanding of the functional complexity of the CNS and will have an immediate and substantial impact on developing effective therapies.

About the Author

Jiaqian Wu, Ph.D. is an assistant professor in the Department of Neurosurgery and Center for Stem Cell and Regenerative Medicine at the University of Texas Medical School at Houston. She did her PhD training at Baylor College of Medicine and postdoctoral work at Yale and Stanford University, focusing on gene expression and transcriptional regulation. She is a recipient of the NIH Pathway to Independence Award (K99/R00) and the Senator Lloyd & B.A. Bentsen Center for Stroke Research award.

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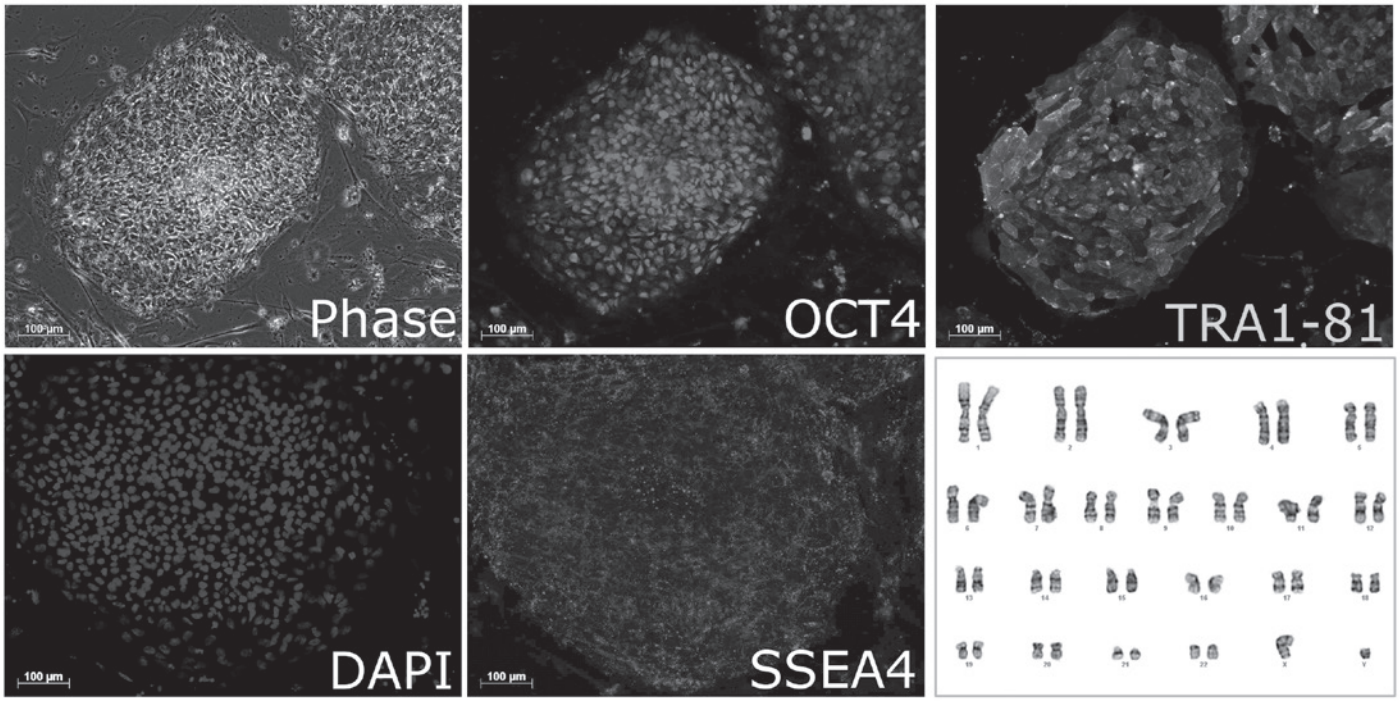

iPSCs reprogrammed from patient urine cells express pluripotent markers and maintain a normal karyotype. The iPSC colonies are tightly packed with distinct margins under phase contrast light microscopy. They also express pluripotent markers such as OCT4, SSEA4 and TRA1-81, which are commonly used to identify iPSCs from other cell populations. The nuclei are counterstained with DAPI.

requirements aimed at translating these cells from bench to the bedside. A major goal of providing clinically safe neural derivatives is a prerequisite for clinical application of iPSCs. Four conditions are necessary. First, any iPSC derivatives must be manufactured within Good Manufacturing Practices (GMP) or Good Laboratory Practice (GLP)-approved facilities, as these are important minimum requirements from the FDA for manufacturing products or drugs for patients. Second, the cells to be grafted must be pre-differentiated and homogeneous, a prerequisite for them to behave as predicted and give rise to the desired cell types and predicted numbers of derivatives in vivo. Third, these cells should be autologous from the same individual, removing the need for immune suppression as would be required for the use of grafts generated from non-self-donors. Finally and most importantly, these cells must either show neuroprotective effects, the ability to improve axon regeneration, or to restore locomotor functions. To meet these requirements, one must first identify the

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appropriate cell types for transplantation, optimize the differentiation protocols, and provide proof of principle in animal models.

Various sources of somatic cells have been tested for reprogramming. Skin-derived fibroblasts are probably the most popular cell type for iPSC generation. However, a skin punch is an invasive procedure, and propagation time is lengthy. To circumvent this problem and expedite the reprogramming process, we have tested cells that were isolated from patients' urine and generated multiple iPSC lines (Figure). The entire process was efficient and posed no harm to the patients. For these reasons, we have begun testing these cells for their therapeutic use in spinal cord injury. The iPSCs gave rise to neural progenitors (identified as A2B5+ cells) and were then grafted into the contused adult mouse thoracic spinal cord at eight days post-injury. Eight weeks after transplantation, the grafted cells were shown to have survived and integrated into the injured spinal cord. These cells expressed neurofilaments and glial fibrillary acidic protein (GFAP), suggesting they had differentiated into neurons and glia, and significantly decreased the lesion size compared to control groups. We are currently testing whether the transplantation of these cells improves locomotor function using a battery of motor assessment tools. We will also test whether these grafts cause unwanted plasticity as evidenced by aberrant synaptic connectivity of pain afferents, or lead to the development or exacerbation of post-traumatic neuropathic pain. Because these cells were able to survive, integrate, migrate and differentiate into host tissue, this process could have broad clinical applications in cell replacement therapy in CNS repair and restoration. The availability of human iPSC-derived, purified neural populations will allow us to tease out the effects of individual neural cell type grafts and combinations of different neural populations.

Purified astrocytes derived from human iPSCs as a promising population for treating SCI

Astrocytes are a major cellular constituent in the CNS and have been shown to critically contribute to neuronal survival, synaptogenesis, neurovascular interaction, and the development of cognition. On the other hand, astrocytes are a known contributor to long-term deficits after spinal cord injury. Numerous reports have shown that mature astrocytes proliferate and create a scar (gliosis) which serves as an impediment to axonal regeneration, preventing remyelination as well as contributing to axon damage. However, recent reports from animal studies have shown that astrocytes are an extremely heterogeneous population with young astrocytes or astrocyte progenitors having the potential to promote both neuroprotection as well as axonal regeneration following SCI. Because the use of astrocyte grafts derived from human cells as a therapeutic strategy remains under explored, we have begun to identify an astrocyte/astrocyte progenitor population that might be beneficial in neuroprotection and regeneration for SCI patients.

Using a combined platform of iPSC and genetic engineering methods, we will soon be able to characterize functional properties and molecular signatures of astrocyte progenitors in vitro, and test whether grafting of astrocyte progenitors will promote functional recovery in both models of acute and chronic SCI by assaying neuroprotection, axonal regeneration and behavioral outcomes (locomotor and neurosensory). Such a human iPSC-based platform offers a reliable and renewable human cell source of defined young astrocyte/astrocyte progenitor population, which are autologous to patients. Using these advanced techniques, there are tremendous implications for possible therapeutic interventions following CNS damage.

Development of CRISPR/Cas9 lineage reporters for differentiation and purification of transcription factor-defined neural progenitors from human iPSCs.

The ability to remove, insert or modify a gene or gene fragment precisely at a specific location in the genome of pluripotent stem cells greatly facilitates the applications of human iPSCs. However, efficiency of such molecular procedures is extremely low in human iPSCs and making genetically modified iPSCs is both time-consuming and labor-intensive. Recently, we have adapted the cutting-edge genome editing technique CRISPR/Cas9 (Clustered Regularly Interspaced Short Palindromic Repeats, CRISPR-associated)-mediated gene targeting platform in the lab. This highly efficient platform has allowed us to quickly obtain multiple genetically labelled-fluorescent protein reporter cell lines for differentiation into defined and purified populations of the neural lineage, including reporter cell lines for neural stem cell, motor neurons, oligodendrocytes, and astrocytes. Overall, CRISPR/Cas9-editing coupled with cell purification approach in a lineage reporter platform during stem cell differentiation should be broadly applicable in any stem cell derivatives and subpopulations of any lineages.

About the Author

Ying Liu, Ph.D., is an assistant professor of neurosurgery at the University of Texas Health Science Center at Houston. She holds a joint appointment at the Center for Stem Cell and Regenerative Medicine, at the UTHealth Brown Foundation Institute of Molecular Medicine. She is also an investigator at Senator Lloyd and B.A. Bentsen Center for Stroke Research. Her research focuses on developing technology platforms for the treatment of spinal cord injury and stroke.

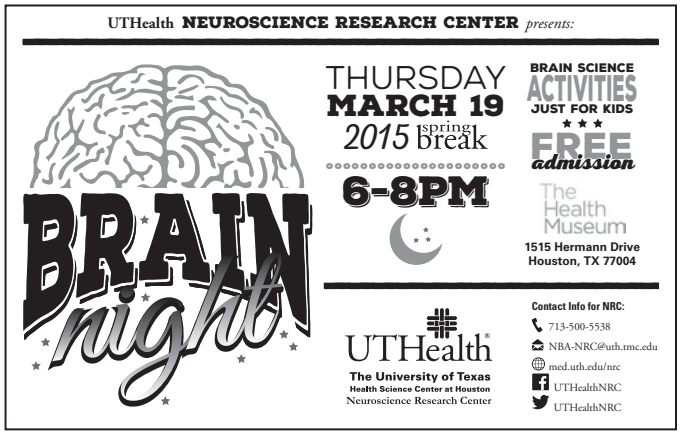
In*the*Spotlight

The NRC Reception at the annual meeting of the Society for Neuroscience, Washington, DC, was held at Hill Country BBQ. This casual event brings together past and present UTHealth NRC members, grad students and postdocs to provide a means of promoting collaborations and scientific exchange.

Upcoming Events

Brain Night at The Health Museum:
Thursday, March 19, 2015, 6:00 to 8:00 pm

The Health Museum, 151 Hermann Drive, Houston, TX. Free event for children and their families; open to the public.



Public Forum: "The Brain on Drugs"
Saturday, April 11, 2015, 10:30 am to Noon

Cooley University Life Center, 7440 Cambridge St., Houston, TX . This free, educational event will be moderated by Dr. Joy Schmitz , Director of the Center for Neurobehavioral Research on Addictions and Professor of Psychiatry and Behavioral Sciences at UTHealth. The event is open to the public and registration is available online through our website.

3rd Annual UTHealth Stomp Out Stroke Festival:
Saturday, May 2, 2015
9:00 am-3:00 pm

Discovery Green, 1500 McKinney St., Houston, TX. This a family friendly event, open to the public, free admission featuring stroke education, free health screenings, nutrition & fitness education, Q&A sessions with physicians, nurses, pharmacists, therapists, family entertainment, music, food and family activities. Our goal is that every Houstonian knows the signs and symptoms of stroke, their stroke risk and how they can reduce that risk. Please contact Dr. Elizabeth Noser at Elizabeth.noser@uth.tmc.edu if you would like to volunteer or for further information.

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because of the continued support and generosity of
individuals in the community.

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Check out the Neurofax calendar of neuroscience events online!

The Neurofax includes seminars, grand rounds, research colloquia, symposia, and local or national conferences that are sponsored by UTHealth, the Texas Medical Center, and other Houston area universities and research institutions. To submit your event to this calendar, please send an email to **nba-nrc@uth.tmc.edu** and include the Event Name, Contact, Date, Time and Location.

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The Neuroscience Research Center Newsletter
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Design: Jeanie Galvanni, UT Printing & Media Services

The Neuroscience Research Center
a Component of
The University of Texas Health Science Center at Houston

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