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Innovative Genetic and Cellular Techniques Help Identify Multiple Disease Targets

CRISPR-Cas9 and iPSC advances hold treatment promise for schizophrenia, addiction, Zika infection and other diseases

WASHINGTON, D.C. — Research released today highlights advances in the use of CRISPR-Cas9 and human induced pluripotent stem cell technologies to identify novel therapeutic targets for neurological disorders such as schizophrenia and addiction. The studies were presented at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

CRISPR-Cas9 is a versatile and highly accurate gene-editing technology that allows researchers to modify specific parts of an organism's genome by altering sections of the DNA sequence. Human induced pluripotent stem cells (iPSCs) are a genetic research tool that can be generated from adult human cells, bypassing the need for using tissue from embryos (along with the associated controversy). The stem cells can be converted into any type of cell in the body, enabling research in multiple human systems. New applications of these relatively recent technologies are facilitating research at the gene-specific level, creating potential for the development of new therapies.

Today's new findings show that:

- An adaptation of CRISPR-Cas9 technology can pinpoint epigenetic "off" and "on" signals for genes that drive cocaine addiction in mice (Peter James Hamilton, abstract 017.01, see attached summary).
- Scientists used CRISPR-Cas9 to shed light on why people with 15q13.3 microdeletion syndrome — a rare human genetic disorder — are more likely to develop brain disorders like autism spectrum disorder, epilepsy and schizophrenia (Karun K. Singh, abstract 103.05, see attached summary).
- Using iPSCs, researchers developed a novel cellular disease model to probe the neurobiological causes of schizophrenia, which are not well understood (ChangHui Pak, abstract 032.29, see attached summary).

Other recent findings discussed show that:

- Using human fetal "mini-brains" grown in 3-D cultures, scientists determined that a specific protein produced by the Zika virus changes the properties of neural stem cells in the developing brain of an infected fetus, potentially causing microcephaly in newborns (Ki-Jun Yoon, abstract 103.06, see attached summary).

"Today's findings exemplify the many advances we've made in using CRISPR-Cas9 and human induced pluripotent stem cell technologies and the amazing discoveries that have resulted," said Hideyuki Okano, MD, PhD, of the Keio University School of Medicine in Tokyo, Japan. "Neuroscientists are using these new gene-editing and molecular tools to develop potential therapeutic targets across multiple disease fronts."

This research was supported by national funding agencies such as the National Institutes of Health, as well as other public, private, and philanthropic organizations worldwide. Find out more about CRISPR-Cas9 and iPSCs on *BrainFacts.org*.

Related Neuroscience 2017 Presentation
Techniques: Exciting New Tools and Technology Emerging from BRAIN Initiative
Tuesday, Nov. 14, 8:30–11 a.m., WCC Ballroom C

Abstract 017.01 Summary

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New Tool Helps Researchers Pinpoint Epigenetic Changes That Worsen Addiction

CRISPR-Cas9-based technology enables exploration of therapeutic targets

Building upon CRISPR-Cas9 gene-editing technology, researchers created a tool that helped them identify a key epigenetic mechanism that drives drug addiction in mice, according to a study released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Advances in developing effective therapies to treat addiction have been stymied by the complex molecular alterations that take place in brains affected by chronic drug exposure. Epigenetic mechanisms in the brain — in which molecular "switches" such as proteins turn genes on and off without changing the underlying DNA sequence — are strongly associated with the progression of addiction. Researchers must understand which epigenetic changes lead to addiction, and which do not, in order to produce targeted therapies for addiction.

In this study, researchers used a novel technical approach to test whether the CREB protein, long thought to be a key molecular mechanism of addiction, drives epigenetic changes in the brain that lead to cocaine addiction in mice. By repurposing the CRISPR-Cas9 gene-editing process, researchers developed a molecular tool that can be directed to nearly any region of the genome. Much like air traffic controllers guide a plane to a precise gate on a specific runway, researchers used their tool to guide CREB into neurons in parts of the mouse brain that process pleasure and reward. The protein acted as an "on-switch" for genes previously linked to drug addiction and caused the mice to seek out cocaine more frequently.

"Our findings illuminate which genes CREB turns on and how this gene activation contributes to the progression of drug addiction," said lead author Peter Hamilton, a researcher at the Icahn School of Medicine at Mount Sinai. "The findings also show promise for confirming the technical validity of these new tools."

Researchers say having a method to test whether specific proteins such as CREB drive addiction may lead to more targeted, and possibly more efficacious, therapies. In addition, similar tools may help uncover other epigenetic or transcriptional events that contribute to neuropsychiatric illness.

Research was supported with funds from the National Institute on Drug Abuse.

Scientific Presentation: Saturday, Nov. 11, 1–1:15 p.m., WCC 144A

Abstract 5731. Engineering CRISPR/Cas9 constructs to model the epigenetic and transcriptional phenomena underlying pathogenic mechanisms of cocaine abuse
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TECHNICAL ABSTRACT: Drug addiction is a chronic, debilitating syndrome with a substantial body of evidence indicating that epigenetic and transcriptional mechanisms are associated with disease progression. However, a major obstacle in efforts to understand and devise treatments for addiction stem from an inability to determine causality between enrichment of an epigenetic modification or transcription factor binding at a specific gene and the pathogenesis of addiction. Only relatively recently has it become possible to target a given type of epigenetic remodeling to a single gene of interest, in order to probe the causal relationship between such regulation and neuropsychiatric disease (Heller et al., Nat Neurosci, 2014; Heller, Hamilton, et al., J Neurosci, 2016). Our group has successfully utilized synthetic zinc-finger proteins (ZFPs) fused to epigenetic editing moieties to determine the neural and behavioral effects of targeted in vivo epigenetic reprogramming in a locus-specific and cell-type specific manner. Given the success of our ZFP approaches, we have broadened our technical repertoire to include the more flexible CRISPR/Cas9 technology. We have designed a fusion construct linking the nuclease-dead Cas9 (dCas9) moiety to a pseudo-phosphorylated isoform of the transcription factor CREB (dCas9-CREB(S133D)) and designed guide RNAs (gRNAs) to target the Fosb gene locus, a locus heavily implicated in the pathogenesis of drug abuse. CREB binding to the promoter of Fosb gene has been demonstrated to underlie the cocaine-mediated induction of ΔFosB. We observe that viral delivery and targeting of dCas9-CREB(S133D) to the Fosb promoter is sufficient to up-regulate ΔFosB mRNA and protein levels in the nucleus accumbens (NAc) of mice as well as potentiate cocaine conditioned place preference, indicating a causal role for CREB binding to Fosb in the progression of cocaine responses. Having utilized these tools at the well-understood Fosb locus, we are now able to design gRNAs targeting CREB to novel loci to understand their causal relevance in the pathogenesis of addiction and other syndromes. The CREB-regulated gene Zfp189 is a promising novel candidate in that it is induced in NAc by cocaine self-administration. The targeted recruitment of CREB to the Zfp189 locus will allow us to identify the causal transcriptional and behavioral consequences of this interaction within the brain's reward regions.

Abstract 103.05 Summary

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Scientists Identify Gene That May Play a Role in Development of Certain Brain Disorders

Discovery may provide insight into the causes of autism spectrum disorder, epilepsy and schizophrenia

Research released today sheds new light on why people with 15q13.3 microdeletion syndrome — a rare human genetic disorder in which 10 genes are deleted on chromosome 15 — are more likely to develop brain disorders such as autism spectrum disorder (ASD), epilepsy, and schizophrenia. One of the genes in the disorder, OTUD7A, plays a unique role in brain development and function, according to a study released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

To determine which of the 10 deleted genes in 15q13.3 microdeletion syndrome causes brain deficits, researchers looked for similar genetic mutations in individuals with ASD, a neurodevelopmental disorder with a strong genetic basis. They analyzed the entire genomic sequence of individuals with ASD, as well as those of family members, and found that of the 10 genes, mutations in OTUD7A were most common. This suggests that when the gene malfunctions or is missing, it has an adverse effect on the brain.

OTUD7A was also the only gene present in large amounts in the human brain compared to other body tissues. "These findings suggests that OTUD7A has a very specific and important function in the brain," said lead author Karun K. Singh, an assistant professor in the Department of Biochemistry and Biomedical Sciences at McMaster University.

Next, the researchers used the gene-editing technology CRISPR-Cas9 to insert OTUD7A into the brain cells of mice that were also missing the 10 genes in 15q13.3 microdeletion syndrome. Before inserting OTUD7A, these genetically engineered mice had neurons that were underdeveloped compared to normal mice, but after researchers reintroduced the OTUD7A gene, the rodents' neurons matured at a normal rate.

This is the first study in which OTUD7A has been implicated as a contributor to the brain deficits associated with 15q13.3 deletion syndrome. Future studies could potentially use CRISPR-Cas9 gene editing to remove OTUD7A in healthy neurons to further examine the gene's role in brain development and function. Such studies could pave the way for novel, targeted treatments, including the potential use of CRISPR-Cas9 techniques to stimulate the remaining copy of OTUD7A in individuals with a 15q13.3 microdeletion to boost its function, which the lab is now testing.

The findings also suggest that the combined genetic and neuroscientific techniques researchers developed to isolate OTUD7A can also be applied to identify disease-causing genes in other neurological conditions caused by the loss of genes — an approach that opens the door to the development of new therapies for other rare genetic disorders.

Research was supported with funds from Canadian Institute of Health Research, NSERC, and the Ontario Brain Institute-POND Platform.

Scientific Presentation: Sunday, Nov. 12, 9–9:15 a.m., WCC 152A

Abstract 5269. Elucidating the pathophysiology of the 15q13.3 micro deletion syndrome
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TECHNICAL ABSTRACT: Copy number variations (CNVs) are chromosomal deletions or duplications that confer high risk for many neuropsychiatric conditions. The 15q13.3 CNV microdeletion is associated with high risk for epilepsy, intellectual disability, schizophrenia and autism spectrum disorder (ASD). However, the neurodevelopmental abnormalities underlying the clinical phenotypes and the gene(s) driving these phenotypes are unknown. To study this CNV, we are utilizing a heterozygous 15q13.3 microdeletion mouse model and patient-derived IPS cells. RNA-sequencing and gene set enrichment analysis (GSEA) of cortical brain tissue from WT and heterozygous mice revealed that differentially expressed genes are highly enriched in forebrain development. We analyzed neuronal morphology, which revealed alterations in dendritic arborization and dendritic spine morphology in excitatory cortical neurons. This is accompanied by alterations in neural activity using patch-clamp electrophysiology. To understand the pathophysiology of 15q13.3 microdeletion syndrome, we dissected candidate driver genes using whole-genome sequencing (WGS) and brain-critical exon analysis of human transcriptome data. WGS of ASD quartet families identified *De Novo* variants in one of the genes within

the deletion, OTUD7A. OTUD7A is also the only gene within the deletion containing a brain-critical exon and is brain-enriched. GSEA of human protein expression data revealed that genes that are highly co-expressed with OTUD7A are highly enriched in pathways involved in synaptic connectivity. Additionally, preliminary experiments showed that OTUD7A is localized to the post-synaptic density of neurons supporting a role for OTUD7A in synaptic connectivity. Current approaches include examining the role of OTUD7A in patient-derived IPSC-neurons, as well as WT IPSC-neurons lacking OTUD7A using CRISPR/Cas9 gene editing. Together, our data suggest OTUD7A is a novel driver gene of the 15q13.3 microdeletion syndrome.

Abstract 032.29 Summary

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New Stem-Cell Model Helps Researchers Probe Schizophrenia's Origins

Analysis tool holds promise for developing therapies that treat cause, not symptoms

Using human-induced pluripotent stem cells (iPSCs), researchers produced neurons with a genetic mutation that increases schizophrenia risk tenfold and compared them to healthy cells, establishing a novel technique for probing the neurobiological causes of this severe mental disorder. The study was released today at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Approximately 1 percent of the global population suffers from schizophrenia, a disease which causes people to have thoughts and hallucinations inconsistent with reality and cognitive problems such as memory deficits. Because schizophrenia's causes are unknown, current treatments are limited to alleviating symptoms rather than addressing the disease at its root.

In this study, researchers focused on synaptic dysfunction — abnormal communication between neurons — as a potential mechanism for the brain disorder. Many genetic mutations slightly increase the risk of schizophrenia, but only one, mutation of a gene called neurexin-1, has been shown to confer a tenfold higher schizophrenia risk in people who carry it. Because the neurexin-1 gene is essential for proper synapse formation and communication between neurons, scientists suspect these functions are key to the disorder.

To probe neurexin-1's role, the researchers obtained iPSCs — human cells that can be reprogrammed into many different cell types — from schizophrenic people with and without the mutation, and from people without schizophrenia. The researchers coaxed the cells to develop into neurons and then analyzed how the cells grew and transmitted electrical signals.

"Using human induced pluripotent stem cells, we were able to generate and analyze neurons from patients with schizophrenia who carry neurexin-1 deletions and compare to those who do not have the disease or the deletion," said lead author Changhui Pak of Stanford University. "Examining how these neurons grow, mature, and communicate with one another will shed light on the pathogenesis of this illness."

The research is still ongoing, but the team hopes that the novel technique will reveal new insight about what causes schizophrenia and that iPSCs-derived neurons could also provide a useful screening tool for new drugs. The study is supported with funds from the National Institute of Mental Health.

Scientific Presentation: Saturday, Nov. 11, 1–2 p.m., WCC Halls A–C

Abstract 8637. Systematic analysis of schizophrenia-associated NRXN1 deletions using human pluripotent stem cell derived induced neurons
C. PAK, S. GRIEDER, T. DANKO, A. HUANG, J. TROTTER, B. ARONOW, A. E. URBAN, D. F. LEVINSON, M. WERNIG, T. C. SUDHOF;
Stanford Univ., Stanford, CA

TECHNICAL ABSTRACT: Synaptic cell adhesion molecules mediate the physical and functional bridging of neuronal synaptic junctions. Neurexin-1 (NRXN1) is a presynaptic cell adhesion molecule that is essential for proper synapse formation and connectivity, and loss-of-function mutations in the human NRXN1 strongly correlate with Autism Spectrum Disorders (ASDs) and schizophrenia (SZ). Recently, we have shown that in human induced neurons bearing conditional heterozygous mutations in NRXN1 results in a specific deficit in the excitatory synaptic strength and neurotransmitter release probability, and in parallel, an up-regulation of calcium/calmodulin-dependent serine protein kinase (CASK) protein level. To investigate the functional relevance of these identified phenotypes in the context of neuropsychiatric disease, we have obtained, differentiated and analyzed a cohort of human induced pluripotent stem cells (iPSCs) from age and gender matched SZ patients carrying NRXN1 deletions and healthy controls. Using the Neurogenin-2 induced neuronal protocol, which generates homogenous populations of cortical glutamatergic neurons, we are able compare functional differences in neuronal morphology, synaptic transmission, and gene expression with homogeneity and reproducibility.

Abstract 103.06 Summary

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Researchers Identify Zika Virus Protein That May Cause Microcephaly

Using mouse and human IPSC 'mini-brain' models, scientists learn how Zika disrupts brain development

Scientists have identified a specific protein produced by the Zika virus that may potentially cause the "small brains" of newborn babies. The protein, NS2A (non-structural protein 2A), changes how neural stem cells divide and mature in the developing brain of an infected fetus, according to recently published research presented at Neuroscience 2017, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Widespread infection by the mosquito-borne Zika virus has become a serious public health concern. Zika virus can spread from a pregnant woman to her fetus, frequently resulting in birth defects such as microcephaly, a condition where the brain does not develop properly, resulting in a smaller-than-normal head.

During embryonic brain development in a healthy fetus, the overall number of neural stem cells is maintained through repetitive cell divisions and proper differentiation — only at the end of brain development do these embryonic cells transform into specialized brain cells. Neural stem cells can lose their capability to divide if the cellular structures that hold them together, called adherens junctions, are disrupted. Scientists have previously reported that Zika virus directly infects neural stem cells and impairs their proliferation, but exactly which component of the virus interacts with the host's molecular machinery is unknown.

To investigate this question, researchers systematically introduced 10 individual proteins generated by Zika virus into neural stem cells of the developing mouse brain. The team found that NS2A, a single protein of Zika virus, leads to reduced proliferation and premature differentiation of neural stem cells in the embryonic mouse brain. The protein also destabilizes the adherens junctions that hold neural stem cells together and allow them to divide properly. When the researchers introduced NS2A to developing human fetal "mini-brains" grown in 3-D cultures, they found that the protein has similar microcephaly-related effects in human brain tissue.

"These results not only reveal a critical component of Zika virus responsible for microcephaly-related effects, but also illuminate a previously unidentified mechanism for how Zika impacts neural stem cell properties," said lead author Ki-Jun Yoon, who conducted the research when he was a postdoctoral fellow at Johns Hopkins University. Understanding the mechanisms underlying the development and progress of Zika virus in the developing mammalian brain may reveal potential targets for therapeutic interventions in the future.

This work was supported with funds from the National Institutes of Health, the Simons Foundation, the Maryland Stem Cell Research Fund, and the Brain & Behavior Research Foundation.

Scientific Presentation: Sunday, Nov. 12, 9:15-9:30 a.m., WCC 202B

Abstract 14097. **K.-J. YOON**[1], G. SONG[2], X. QIAN[2], J. PAN[3], D. XU[4], H.-S. RHO[5], F. ZHANG[6], E. LEE[8], Q.-F. WU[4], K. M. CHRISTIAN[10], H. TANG[9], P. JIN[7], Z. XU[4], J. QIAN[11], H. ZHU[12], H. SONG[10,13], G.-L. MING[14,13];
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TECHNICAL ABSTRACT: Zika virus (ZIKV) directly infects neural progenitors and causes proliferation deficits. However, the way in which ZIKV interacts with the host machinery to impact neurogenesis in the developing mammalian brain is not well understood. To reveal the critical components that induce the ZIKV pathogenesis linked with microcephaly, we cloned 10 open reading frames from the ZIKV genome into an expression vector, which we used to introduce individual proteins into radial glial neural stem cells in the embryonic mouse cortex by in utero electroporation. As a result, we identified two ZIKV-encoded proteins that affect the proliferation and maintenance of radial glial cells. To investigate how ZIKV proteins directly interact with the host machinery to impact neural stem cell behavior, we performed a protein microarray assay to screen for human proteins that can bind to ZIKV proteins in vitro in an unbiased manner. We identified host interacting protein candidates that are essential for neural progenitor functions and validated the binding in cultured mouse neural stem cells using a co-immunoprecipitation assay. Next, we observed that expression of a candidate ZIKV coding component in human forebrain organoids led to reduced radial glial cell proliferation and deficits in radial glia fiber scaffolding, resembling postmortem forebrain tissue of the first reported ZIKV-infected microcephalic fetus from an infected mother. Together, our results reveal novel pathogenic mechanisms underlying ZIKV infection in the developing mammalian brain.