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Harnessing the Power of Glia and Stem Cells

Findings underscore the importance of the fast-growing fields of glial and stem cell research

CHICAGO — Research presented today demonstrates scientists' evolving understanding of the role of glial cells and the utility of stem cells in the study neurological conditions. Glia are non-neuronal cells that support and protect neurons, but new findings reveal how these cells also influence brain activity and can be reprogrammed to generate new neurons in the diseased brain. New research also shows how scientists are using stem cells to create a model of Down syndrome that allows them to better study the disease. The research was presented at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. These findings have potential implications for the treatment of neurological disorders, such as addiction, Down syndrome, and stroke.

Today's new findings show that:

- Increasing the expression of a single protein, NeuroD1, in the brain tissue of stroke-injured mice can reprogram glial cells to become functional neurons (Yuchen Chen, abstract 193.07, see attached summary).
- New neurons can be generated from glia in the brains of healthy young mice by increasing the joint expression of two proteins, Ascl1 and Sox 2 (Sophie Peron, abstract 193.02, see attached summary).
- Disruptions in the release of the neurotransmitter glutamate from glial cells called astrocytes can lead to compulsive behavior in rats, including behaviors associated with addiction and OCD (Evan Hess, abstract 411.20, see attached summary).
- Using a Down syndrome tridimensional mini-brain model (cerebral organoids) generated from human stem cells, researchers reveal that an extra copy of chromosome 21 leads to aberrant protein expression (Tristan McClure-Begley, abstract 202.09, see attached summary).

"These exciting findings are changing our understanding of the roles, importance, and potential uses of glia and stem cells," said Carol Marchetto, PhD, of the Salk Institute for Biological Sciences. "The molecular biology of the brain is incredibly complex, and we need as many tools as possible to unravel that complexity."

This research was supported by national funding agencies such as the National Institutes of Health, as well as other private and philanthropic organizations. Find out more about the latest research involving glial and stem cells at *BrainFacts.org*.

Related Neuroscience 2015 Presentation:
Human iPSC-Derived Cells for Modeling Neurodegenerative Disease and Drug Discovery
Sunday, Oct. 18, 1:30-4 p.m., S100A

Abstract 193.07 Summary

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Increased Expression of Specific Protein Turns Glial Cells Into Functioning Neurons in Brain Tissue of Mice

Research may lead to new therapies for reducing stroke-related brain damage

By increasing the expression of a single protein scientists have created new neurons from "support" cells known as glia in the brain tissue of stroke-injured mice, according to research released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. This finding has important implications for the treatment of stroke, which kills an estimated 6 million people worldwide each year and leaves about 5 million permanently disabled.

"The current effective treatment for stroke needs to be applied within a few hours after symptoms first appear," said lead author Yuchen Chen of Penn State University. "Many people do not receive the treatment in time and, as a result, suffer from secondary brain damage. We urgently need more effective therapies to reduce the brain damage of stroke patients and help them recover faster from disabilities."

When a stroke occurs, neurons (the functional brain cells that process information) often die or degenerate, while glia (the supportive brain cells that help neurons communicate) become activated and enlarged. Initially, the glial cells create a defensive barrier to protect healthy neurons from dying, but that barrier eventually becomes a glial "scar," which limits regrowth of neurons and hinders recovery.

To determine whether the harmful effects of stroke-induced glial scars could be reversed, Chen and colleagues injected viruses that express a protein known as NeuroD1 into tissue from the cerebral cortices of stroke-injured mice. By binding to specific DNA sequences, NeuroD1 activates genes that turn glial cells into neuronal cells. The cerebral cortex is the brain's main processing center and plays a key role in movement, sensation, and language — all functions often permanently damaged by stroke. The direct conversion of reactive glial cells into neurons generates several significant effects in the cortical tissue: The glial scar becomes much smaller, neuronal loss is greatly reduced, and cortical tissue is largely preserved.

"These findings suggest that direct reprogramming of glial cells into functional neurons may provide a completely new approach for brain repair after stroke," Chen said. "Our next step is to analyze whether the glia-neuron conversion technology can facilitate functional recovery in stroke animals."

Research was supported with funds from the National Institutes of Health and the Penn State University Endowment Fund.

Scientific Presentation: Sunday, Oct. 18, 1-3 p.m., N426 A

193.07, Rebuilding mouse cortex after ischemic stroke by *in situ* reprogramming reactive astrocytes into functional neurons
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TECHNICAL ABSTRACT: Our recent studies have demonstrated that overexpression of single transcription factor NeuroD1 enables direct reprogramming of reactive astrocytes into functional neurons, which can successfully integrate into the local neural circuits in adult mouse cortex (Guo et al., 2014, Cell Stem Cell). Our in vivo cell conversion technology makes it possible to develop a novel regenerative therapy to treat brain injury and neurodegenerative disorders, especially those with severe loss of neurons. Brain ischemic stroke is a major cause of death and disability, and there is no effective therapy currently available to restore neuronal loss or recover tissue damage after cerebral ischemia. After stroke, the injury core area is occupied with reactive astrocytes, which is a major obstacle for neuron functional recovery. Here we report an innovative approach to reprogram reactive astrocytes directly into functional neurons after stroke in order to restore the lost neuronal functions. We employed a mouse focal ischemic stroke model by injecting endothelin-1 into mouse cortex to cause blood vessel constriction and brain damage. After stroke, we regenerated functional neurons by infecting astrocytes with adeno-associated virus (AAV) expressing NeuroD1. Electrophysiological analysis demonstrated that the NeuroD1-converted neurons were functional in forming synaptic connections with other neurons. CTB retrograde tracing showed that the NeuroD1-converted neurons could project to long-range target regions. Intriguingly, we found great beneficial effects brought by NeuroD1-mediated glia-to-neuron conversion in the stroke area: (1) the cortical atrophy was significantly reversed, (2) loss of neuronal marker NeuN was greatly reduced, (3) the glial scar formation by reactive astrocytes and microglia was also significantly reduced. Taken together, our studies suggest that direct reprogramming of reactive astrocytes into functional neurons will provide a potential therapy for brain repair after stroke.

Abstract 193.02 Summary

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Co-Expression of Two Proteins Turns Glial Cells Into Neurons in Mice

Finding supports the theory that glia-neuron conversion may lead to effective therapies for some brain disorders

New neurons can be generated in the healthy brains of young mice by forcing supportive cells known as glia to increase the joint expression of two proteins, according to research released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. This finding adds to the growing evidence that glia-to-neuron conversion therapies are promising strategies for treating brain diseases and injuries that result in a loss of neurons.

Recent research has shown that glial cells can be converted into neurons by forcing them to express regulatory proteins called transcription factors. While these proteins regulate nuclear gene expression and neurogenesis during development, when overexpressed in glial cells, they induce cellular reprogramming, namely the switch of glia to neuronal cells.

For the study, the researchers forced glial cells to express two proteins, Ascl1 and Neurog2. Both had previously been shown to independently convert glial cells into early version of neurons known as neuroblasts. Those experiments had been conducted on brain tissue in Petri dishes, however. For this new study, the same proteins were overexpressed in vivo — in the brains of mice — but very little glia-to-neuron conversion occurred. The researchers then decided to force one of the proteins, Ascl1, to overexpress in vivo together with Sox 2, a protein that plays a key role in the maintenance of both embryonic and adult stem cells. The joint expression of Ascl1 and Sox 2 resulted in the creation of new neuroblasts.

"Our research shows that glial cells can be converted into neurons in healthy mice as well as in mice with brain injuries — a further confirmation of the potential value of this reprogramming process," said lead author Sophie Peron at the Johannes Gutenberg University in Mainz, Germany. "Next, we need to identify strategies for making the reprogramming more efficient, and then to see if these newly generated neurons can restore function."

Research was supported with funds from the Deutsche Forschungsgemeinschaft and the Belgian Science Policy Office.

Scientific Presentation: Sunday, Oct. 18, 1-3 p.m., N426 A

193.02, Direct *In vivo* glia-to-neuron conversion in the postnatal mouse cerebral cortex
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TECHNICAL ABSTRACT: Neuronal loss is common to numerous brain diseases or injuries and is often accompanied with irreversible dysfunctions. One strategy to overcome the limited regeneration capacity of the central nervous system relies on cell replacement based therapies. Among them, direct reprogramming without passing through a pluripotent state of resident brain cells into neurons has been proposed as a new innovative strategy and has gained significant momentum within the last few years. Previous studies from our lab have shown that forced expression of Ascl1 and Neurog2 can direct postnatal cortical astrocytes towards GABAergic and glutamatergic neurogenesis in vitro (Heinrich et al., 2010; Heinrich et al., 2011). In this study, we aim at testing whether glia can be lineage converted in the postnatal mouse cerebral cortex in vivo. Glial cells proliferate locally in the early postnatal cortex (Ge et al., 2012). To target these cells for lineage conversion we transduced proliferating glia with retroviruses encoding for various neurogenic transcription factors. We found that overexpression of the transcription factors Ascl1 and Neurog2 alone in the postnatal cortex in vivo hardly gives rise to doublecortin (DCX) expressing neuroblasts. This suggests that glial cells in vivo are less responsive and that additional obstacles may to be overcome to acquire a neuronal identity. Thus, we tested whether co-expression of Sox2 can facilitate reprogramming induced by Ascl1. Consistent with the previously observed synergism between Sox2 and Ascl1 on lineage reprogramming of adult cells (Karow et al., 2012; Heinrich et al., 2014), we found here that combined expression of these two transcription factors results in the lineage conversion of proliferative glia into DCX positive neuroblasts in the early postnatal cortex in vivo. Together, our work demonstrates for the first time the feasability of direct in vivo lineage reprogramming in the healthy cerebral cortex and emphazises the influence of the in vivo milieu on the susceptibility of glial cells to be directed towards a neuronal identity.

Abstract 411.20 Summary

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Impairment of Chemical Pathway in Brain Cells Linked to Compulsive Behavior in Rats

Finding provides insight into possible pathology behind disorders such as drug addiction and OCD

Disruptions in the release of a chemical messenger from brain cells known as astrocytes can lead to compulsive behaviors in rats, including behaviors associated with addiction and obsessive compulsive disorder (OCD), according to research released today at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. Such disorders, which affect millions of people worldwide, are characterized by a loss of control over one's thoughts or behaviors.

Astrocytes are a type of glial cell, non-neuronal cells that provide support and protection for neurons. Although all their functions are not clearly understood, astrocytes are known to communicate with and regulate neurons through the release of the chemical messenger glutamate, the primary excitatory neurotransmitter in the brain.

"Our research suggests that the release of glutamate from astrocytes is critical for behavioral control," said lead author Evan Hess of Marquette University. "The findings also offer more evidence of the important and complex role that these glial cells perform in the brain."

For their study, Hess and his colleagues conducted a series of experiments in rats that were unable, as a result of genetic engineering, to release glutamate through astrocyte cells in their brains. The experiments involved having the rats learn and then relearn the "rules" of a maze to obtain a food reward. This type of experiment tests cognitive flexibility — the ability to alter thoughts or behaviors in response to a changing environment. Other research has linked poor cognitive flexibility to both addiction and OCD. The current experiments found that rats with an impaired ability to release glutamate were significantly worse at figuring out the rules of a new maze than rats without the glutamate impairment — a sign of reduced cognitive flexibility.

"Neuroscience has traditionally been focused on studying communication between neurons as a means to understanding behavior, but our research suggests that astrocytes can alter communication between neurons and therefore play an active role in maladaptive behaviors that underlie compulsive behaviors, such as addiction and OCD," Hess said. "Our next step is to investigate how a loss of glutamate alters specific brain circuits. The goal is to gain further insight into the pathogenesis of compulsive disorders — and to find new therapies for treating them."

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

Scientific Presentation: Monday, Oct. 19, 4-5 p.m., Hall A

411.20, Maladaptive behaviors resulting from decreased glutamate release from astrocytes: phenotyping a system xc- knockout rat
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TECHNICAL ABSTRACT: Excitatory signaling is achieved by an elaborate network involving reuptake and release mechanisms expressed by both neurons and astrocytes. System xc- (Sxc-) is a non-vesicular glutamate release mechanism primarily expressed by astrocytes that has been shown to exert profound control over multiple aspects of synaptic transmission. Sxc- is expressed in brain regions linked to diverse behaviors such as the basolateral amygdala, bed nucleus of the stria terminalis (BNST), nucleus accumbens (NAc), and medial prefrontal cortex (mPFC). As such, its dysfunction may contribute to CNS diseases ranging from drug addiction to schizophrenia. To determine whether glutamate release from astrocytes is essential for complex behavioral phenotypes, we created transgenic rats lacking Sxc- activity by mutating the Slc7A11 gene using Zinc-Finger Nucleases (ZFN). Genotyping data revealed successful mutations of Slc7A11 in two lines of Sprague Dawley rats. The first line contains a 39 base pair deletion in exon 2 leading to a frameshift and anticipated truncation of the xCT protein. The second line has a deletion of 13 amino acids corresponding to the majority of the 3rd transmembrane domain. The deletion of Sxc- function was verified in each line by demonstrating the lack of active 14C-cystine uptake into brain slices or cultured cells. Mutant system xc- (MSxc) rats displayed normal survival rates, growth patterns, and basal levels of activity. However, MSxc rats exhibited significant differences in multiple behavioral assays including elevated plus maze, attentional set shifting, cocaine self-administration/reinstatement, and social interaction. Because many of the behavioral deficits exhibited by MSxc rats may reflect cognitive inflexibility, we have begun to examine key cortical areas for cellular, molecular, and structural abnormalities that could underlie these diverse maladaptive behaviors. To date, we have detected reduced PV expression in the prefrontal cortex and increased PV expression in the sensorimotor cortex. This pattern would be consistent with cognitive inflexibility stemming from an imbalance between goal- and habit-driven behaviors.

Abstract 202.09 Summary

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Stem Cell Model Helps Profile Protein Expression of Down Syndrome Genes

Findings may help unlock important clues about the genetic condition's effects on the brain

Using tissue grown from the stem cells of an individual with Down syndrome, scientists have identified an aberrant expression of proteins that may help explain how the genetic disorder affects the brain. The research was released at Neuroscience 2015, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

Down syndrome, which results when an individual has an extra copy of chromosome 21, affects about 1 in 1,000 births and is one of the most common causes of intellectual disability worldwide. "That third copy of chromosome 21 influences all aspects of embryonic development, including critical steps during brain development," said lead author Tristan McClure-Begley of the University of Colorado-Boulder. "But we've had trouble identifying exactly why the extra chromosome has such widespread effects, partly because we've lacked good human tissue models of Down syndrome."

To address this problem, McClure-Begley and his colleagues generated human brain tissue from two induced pluripotent cell lines derived from an individual with Down syndrome. Induced pluripotent stem cells have the ability to differentiate into any type of cell in the body. One cell line in these experiments contained an extra chromosome 21, but the other did not. Using advanced laboratory techniques, the researchers then compared the two tissues to identify any differences in the amount of proteins expressed. Proteins, which do most of the work in cells, are created (or expressed) from information from genes. The comparison revealed that key proteins are differentially expressed because of the presence of the extra copy of chromosome 21. These results gave the researchers leads on which genes are important players in the neurobiology of Down syndrome.

"This finding helps us better understand how the early development of the central nervous system is affected by Down syndrome," McClure-Begley said. "We're currently looking at the effects of various drugs on these tissue models. Our hope is that this research will lead to interventions that might ameliorate the effects of Down syndrome."

Research was supported with funds from the Linda Crnic Institute at the University of Colorado.

Scientific Presentation: Sunday, Oct. 18, 1-4:15 p.m., N230

202.09, Proteome-wide effects of Down syndrome explored with human induced pluripotent stem cell-derived cerebral organoids
***T. D. MCCLURE-BEGLEY**, M. KLYMKOWSKY, C. E. EBMEIER, K. BALL, W. OLD;
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TECHNICAL ABSTRACT: In order to better understand the cellular and molecular processes associated with Down syndrome (DS) in the human central nervous system (CNS), we generated a model of early neuronal development that capitalizes on the use of human induced pluripotent stem cells (IPSc) as a starting template. We obtained IPSc from an individual where lines had been created that both contained (C2; T21), and had lost the extra copy of chromosome 21 (C2-43; D21). With some modifications to the method first described by Lancaster et al (Nature, 2013), we successfully generated human cerebral organoids from both the C2 and C2-43 cell lines and used them for imaging experiments with whole-mount immunostaining and laser scanning confocal microscopy as well as a deep proteome profile with label-free quantitation of over seven thousand proteins in each sample. Our imaging analysis shows that at the time of our sample preparation, neurons had been generated that populated the outer edges of the tissue, with evidence of populations of radial glia restricted to the inner regions of the tissue; a distribution of cell types indicative of radial migration and differentiation, similar to the development of human cortex. Our proteomics analysis shows many proteins changing in significant abundance due to Trisomy 21, with striking alterations in members of the Wnt and Notch signaling pathways, as well as catecholamine metabolism, axon guidance, and cell adhesion. These early data are the first to demonstrate the utility of IPSc-derived cerebral organoids in the study of complex genetic conditions with a spectrum of neurological phenotypes.