



Embargoed until November 18, 4:30 p.m. ET
Press Room, November 15–19: (202) 249-4125

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RESEARCH REPORTS DISCOVERY OF NEW SOURCES OF NEURAL STEM CELLS

Findings advance progress toward ability to restore cells damaged by stroke, vision and hearing loss, and neurodegenerative disease

Washington, DC — New findings released today report that scientists are uncovering new sources and uses of neural stem cells, including some with the potential to restore cells damaged by disease or disorder. These conditions include stroke, vision loss, hearing loss, and neurodegenerative diseases such as Alzheimer’s disease, Parkinson’s disease, and amyotrophic lateral sclerosis (ALS). The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world’s largest source of emerging news about brain science and health.

These new findings show that:

- A vestigial remnant of the human spinal cord known as the filum terminale offers a new source of adult neural stem cells. The source is not necessary for brain function, and the cells can be reimplanted into the patient from whom they came (Ruchira Jha, abstract 407.7, see attached summary).
- Mesenchymal stem cells derived from human bone marrow have the potential to restore damaged hair cells of the inner ear (Sujeong Jang, abstract 720.1, see attached summary).
- Pluripotent stem cells — those that give rise to almost every type of cell in the body — can be converted into all seven classes of retinal cells that are necessary for vision (Michael Ezra Zuber, abstract 407.13, see attached summary).
- Latent stem cells exist in the hippocampus, an area of the brain essential for memory and learning, and these cells can be activated to produce new neurons (Tara Walker, abstract 523.8, see attached summary).

“These discoveries illustrate stem cell research’s continuing extraordinary potential to treat a wide range of deadly and disabling diseases that affect millions,” said press conference moderator Anand Swaroop, PhD, of the National Eye Institute. “The emerging role of stem cells also highlights how basic research to advance scientific knowledge in turn forms a foundation for health-related discovery, often in unexpected ways.” Swaroop’s laboratory is focusing on generating photoreceptors from stem cells; he expects that early success in stem cell therapy will likely come for retinal and macular degenerative diseases, as the retina is one of the most accessible parts of the central nervous system.

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Abstract 407.7 Summary

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Research Identifies New Source of Adult Stem Cells

Potential for treating spinal injury, Alzheimer's, and Parkinson's disease

Researchers from Harvard University have discovered a new source of adult neural stem/progenitor cells: a vestigial remnant of the human spinal cord known as the filum terminale. These cells may one day prove useful in treating spinal cord injuries and neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (ALS), said Ruchira Jha, the study's lead author. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

For this study, Jha and David Cardozo, PhD, obtained filum terminale from 19 children aged 3 months to 18 years who were having this tissue removed as a part of a therapeutic procedure for a condition called tethered cord syndrome. The filum terminale, like the appendix in the gastrointestinal system, has no essential function and is easily accessible surgically. "From this tissue, we isolated neural stem cells in the form of balls of cells called neurospheres," said Jha. "These cells continued to divide in culture conditions for up to nine months."

The researchers then successfully differentiated the isolated neural stem cells into neural cell types, including neural progenitor cells, neurons (including motor neurons), and glia. By culturing the human neural stem cells with rat muscle cells, they were able to turn the stem cells into motor neurons that innervated the muscle.

Importantly, the source of the cells is not essential for brain function; the cells also are autologous, meaning they can be reimplanted into the same patient from whom they came, reducing rejection risks and other issues associated with nonautologous sources.

"Of course, the road to using these cells for any therapeutic benefit will be an extremely long one," said Jha. "One of the next steps will be to explore the potential of these stem cells to differentiate into specific types of neurons beyond motor neurons, such as the dopamine neurons that are lost in Parkinson's disease."

The research was supported by the Lefler Foundation and Vertex Pharmaceuticals.

Scientific Presentation: Monday, November 17, 2:30–2:45 p.m., Washington Convention Center, Room 147B.

407.7, The human and rat filum terminale- a novel source of multipotent cells

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TECHNICAL ABSTRACT: Neural stem cells (NSCs) are multipotent cells that arise from specific germinative zones in the central nervous system (CNS) and proliferate in response to certain growth factors like epidermal growth factor (EGF) and basic fibroblast growth factor (bFGF). Past research has demonstrated the presence of multipotent NSCs/progenitor cells in regions of the adult mammalian CNS such as the spinal cord, retina, subependymal lining of the ventricles, and subgranular layer of the hippocampus. However, current sources of NSCs are isolated from regions of the CNS that are difficult to access and critical for normal function. This precludes their clinical use for autologous transplantation. Unlike previously isolated NSCs from the mammalian CNS, the filum terminale (FT), a tissue that extends from the conus medullaris and anchors the spinal medulla to the coccyx, is surgically accessible and serves no essential function in humans. It potentially provides an expendable source of autologous human NSCs for therapeutic use. We hypothesized that this may be a potential source of stem cells due to its unique developmental history of 'de-differentiation', propensity for tumor formation, and histological niche.

To test this hypothesis we used two models: rat (rFT) and human (HpFT). FT was identified and dissected from postnatal rats or obtained from pediatric tissue from children undergoing FT excision surgery for tethered cord syndrome. HpFT and rFT tissue sections stained positive for

Nestin. Using growth factors such as EGF, bFGF, and leukemia inhibitory factor (LIF), neurospheres were isolated from cultures of rFT and HpFT (up to 18 years of age) and passaged up to 9 months *in vitro*. When subject to differentiating conditions (adhesive substrate, serum), single FT neurospheres (rFT n= 65; HpFT n= 50) gave rise to multiple cell types including neural progenitor cells, neurons and glia. Incubation with tritiated thymidine demonstrated that differentiated cells originated from actively dividing neurosphere cells. Single neurospheres (rFT n= 25; HpFT n=10) generated motor neurons *in vitro* under controlled conditions. Single neurospheres (rFT n= 24; HpFT n=18) that were treated with motor neuron growth factors, retinoic acid, and sonic hedgehog, and co-cultured with rat myocytes, generated motor neurons that formed neuromuscular junctions *in vitro*. This is an exciting discovery given the potential therapeutic implications for many currently incurable CNS diseases including trauma and degenerative diseases (ALS, Alzheimer's, Parkinson's).

Abstract 720.1 Summary

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Bone Marrow-Derived Stem Cells Show Potential to Restore Damaged Inner Ear Hair Cells

Animal research advances understanding about leading cause of hearing loss worldwide

Mesenchymal stem cells (MSCs) derived from human bone marrow have the potential to restore damaged hair cells of the inner ear, suggests a new study from Chonnam National University in Gwang-ju, South Korea. Such damage is the leading cause of moderate to profound hearing loss, a disability that affects an estimated 278 million people worldwide, according to the World Health Organization. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

“When sensitive hair cells in the inner ear of humans and other mammals are killed — by loud noise, autoimmune attack, toxic drugs, or aging — the damage is permanent,” said Sujeong Jang, MD, who led the study. “Birds and reptiles are luckier. Their damaged hair cells apparently regenerate and can restore normal hearing.”

In recent years, scientists have begun to investigate whether stem cell therapy could do for humans what nature does for birds and reptiles — restore the tens of thousands of hairlike cells that turn sound waves into electrical signals in the organ of Corti, sometimes called the “microphone” of the cochlea, the auditory section of the mammalian inner ear. MSCs offer a promising source of stem cells for this research because they can be obtained from a patient's own bone marrow and differentiate readily into many types of cells, including neurons.

In this study, Jang and his colleagues differentiated human bone marrow-derived MSCs into neuron-like cells and then transplanted those cells into the injured inner ear of guinea pigs. After two to three months, the animals showed evidence of some restored hearing in the treated ears. In addition, a microscopic examination of the animals' treated ear tissue found that the numbers of hair cells in the organ of Corti and in another area of the cochlea known as the spiral ganglia had increased significantly.

“These results suggest that bone marrow-derived MSCs have the potential to restore inner ear hair cells,” said Jang.

The research was supported by the Korea Research Foundation.

Scientific Presentation: Wednesday, November 19, 8–9 a.m., Washington Convention Center, Hall A-C

720.1. Bone marrow-derived mesenchymal stem cells transplantation into injured cochlea accelerates hearing recovery in guinea pigs
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TECHNICAL ABSTRACT: In mammals, sensory hair cell loss resulting from aging, ototoxic drugs, infections, overstimulation and other causes is irreversible and leads to permanent sensorineural hearing loss. We used bone marrow-derived human mesenchymal stem cells (hMSCs) to replace the degenerated hair cells and spiral ganglion neurons. When grown in differentiation media, hMSCs expressed high levels of neural markers, and most of hMSCs showed voltage-dependent sodium currents. Moreover, RT-PCR revealed the molecular evidence of high levels of mRNA for the functional ionic currents. After neural differentiated hMSCs were transplanted into injured inner ear of guinea pigs, numbers of cell body in the organ of Corti and spiral ganglia were increased significantly. Human specific phenotype marker gene mRNAs of sensory hair cells or supporting cells were detected in transplanted inner ear. Furthermore guinea pigs which were taken hMSCs transplantation showed a significant recovery in ABR test. These results suggest that bone marrow-derived hMSCs have the potential to restore inner ear hair cells (This work was supported by the Brain Korea 21 Project, Center for Biomedical Human Resources at Chonnam National University, Korea Research Foundation Grant Funded by the Korean Government (Basic Promotion Fund: KRF-2006-E00379), and Regional Research Centers Program (Biohousing) of the Korean Ministry of Education & Human Resources Development).

Abstract 407.13 Summary

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Pluripotent Stem Cells Shown to Generate New Retinal Cells Necessary for Vision

Promise shown for addressing debilitating vision loss due to a range of causes

Pluripotent stem cells — those, like embryonic stem cells, that give rise to almost every type of cell in the body — can be converted into the different classes of retinal cells necessary for vision, according to a new study from researchers at SUNY Upstate Medical University in Syracuse, N.Y. This research points to exciting new possibilities for preventing or reversing the disabling vision loss caused by age-related macular degeneration, diabetes retinopathy, retinitis pigmentosa, glaucoma, and other diseases that damage the retina, the layer of light-sensitive nerve cells that line the back of the eye. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health. "Vision is lost in these diseases because one or more of the seven retinal cell types die," said Michael Ezra Zuber, PhD, the study's lead author. "Current treatments can slow these diseases' progression, but they can't replace lost retinal cells. Pluripotent cells offer a promising starting point from which to generate new retinal cells."

Zuber and his colleagues knew that cultured pluripotent cells could be induced to express some retinal cell genes, but they didn't know if all retinal cell classes could be generated or if the cells would have the ability to form a functioning retina. To test that hypothesis, the scientists turned to pluripotent *Xenopus laevis* (frog) cells. Under normal conditions, pluripotent frog cells form only skin tissue. The scientists were able, however, to convert the pluripotent cells to retinal cells by forcing them to express the eye field transcription factor (or EFTF) genes. The reprogrammed cells formed all seven classes of retinal cells normally found in the eyes, including the retinal ganglion cells, which have axons (optic nerves) that extend to the brain.

Furthermore, these new cells eventually formed into functioning eyes. When tested, tadpoles used their induced eyes to detect light and to engage in a vision-based behavior. The scientists also found a population of self-renewing cells in the periphery of the induced retinas, suggesting that EFTF-induced cells also formed adult retinal stem cells.

"The goal of regenerative medicine is to replace dead or dying cells," said Zuber. "The retina, like all body organs, contains multiple, distinct cell types. Therefore, successful recovery from blindness due to injury or disease will require the functional replacement of multiple retinal cell types. Our results demonstrate that pluripotent cells can be purposely altered to generate all the functional retinal cell classes necessary for vision."

The research was supported by Research to Prevent Blindness, the E. Matilda Ziegler Foundation for the Blind, The Lions Club of Central New York, and the U.S. National Eye Institute.

Scientific Presentation: Monday, November 17, 4–4:15 p.m., Washington Convention Center, Room 147B

407.13, Generation of retinal stem cells and functional eyes from pluripotent cells

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TECHNICAL ABSTRACT: Pluripotent cells are a potential starting point from which to generate organ specific stem cells. For example, conversion of pluripotent cells to retinal stem cells could provide an opportunity to treat retinal injuries and degenerations. Although pluripotent cells have been induced to express some retinal markers, it is not known if retinal stem cells form, or if induced cells can generate functional retina. We took advantage of the relatively simple *Xenopus laevis* animal cap system to address these questions. Previous work demonstrated that

a group of transcription factors (the eye field transcription factors; EFTFs) are coordinately expressed in the embryonic eye field, form a self-regulating feedback network and are essential for eye formation. Primitive ectoderm cells isolated from the animal pole of blastula stage embryos are pluripotent. When cultured in isolation, then transplanted to developing embryos, the cells differentiate into epidermis. However, if treated with the appropriate inducer, they can form cell types of all three germ layers. Using microarray analysis we found that cultured, EFTF-expressing primitive ectoderm has a transcriptional profile similar to the endogenous eye field. EFTF-expressing cells also appear determined to form retinal tissue since they generate retinal cells and eye-like structures when transplanted to the embryonic flank. We show the cells are self-renewing as they continue to proliferate in the peripheral retina where adult retinal stem cells are normally located. As a test of multipotency, we replaced one of the two endogenous eye fields with EFTF-expressing pluripotent cells. Donor cells differentiated into all seven retinal cell classes of the mature retina, including retinal ganglion cells, which extended axons to the host brain. Using both the electroretinogram and a phototropic behavioral assay we show the eyes that form from induced retinal cells are functional. Since EFTF-expressing pluripotent cells are both multipotent and self-renewing, we have named these cells induced retinal stem cells (iRSCs). Our results demonstrate the fate of pluripotent cells can be purposely altered to generate multipotent retinal stem cells, which differentiate into functional retinal cell classes and form a neural circuitry sufficient for vision.

Abstract 523.8 Summary

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Renewable Source of Stem Cells Identified in the Hippocampus

Animal study shows potential to produce additional neurons

A team of Australian scientists has found a renewable source of stem cells in the hippocampus, an area of the brain essential to memory and learning, and, even more importantly, a way of activating those cells to produce more neurons. The research was presented at Neuroscience 2008, the annual meeting of the Society for Neuroscience and the world's largest source of emerging news about brain science and health.

These findings could lead to new therapies that stimulate the repair or replacement of neurons damaged by neurodegenerative diseases that affect the hippocampus, such as Huntington's disease and stroke, said Tara Walker, PhD, the lead author of the study.

"The loss of neuronal production in the hippocampus characterizes these diseases," said Walker. "But, surprisingly, no previous studies were successful in identifying a resident stem cell population in the hippocampus that was capable of providing the renewable source of these essential nerve cells."

In this study, Walker and her colleagues grew balls of cells (neurospheres) from precursors present among dissociated mouse hippocampal cells. After stimulating these cells with neural activity (potassium-induced depolarization), they found far more neurospheres, including some very large neurospheres not seen before. It was only these very large neurospheres that contained multipotent stem cells that could be differentiated into new neurons.

"Since these latent stem cells are now known to reside in the adult hippocampus, this finding suggests there may be a way of creating new neurons to replace neurons lost to degenerative diseases," said Walker. "In addition, decreased neurogenesis appears to be involved in dementia and depression, so activation of these cells could also provide a frontline therapy for treating these conditions."

Scientific Presentation: Tuesday, November 18, 11 a.m.–noon, Washington Convention Center, Hall A-C

523.8. Latent stem and progenitor cells in the hippocampus are activated by neural excitation

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TECHNICAL ABSTRACT: The regulated production of neurons in the hippocampus throughout life underpins important brain functions such as learning and memory. Surprisingly, however, studies have so far failed to identify a resident hippocampal stem cell capable of providing the renewable source of these neurons. Here we report that depolarizing levels of KCl produce a 3-fold increase in the number of neurospheres generated from the adult mouse hippocampus. Most interestingly, however, depolarizing levels of KCl led to the emergence of a small subpopulation of precursors (approximately 8 per hippocampus) with the capacity to generate very large neurospheres (>250 μ m in diameter). Many of these contained cells which displayed the cardinal properties of stem cells: multipotentiality and self-renewal. In contrast, the same conditions led to the opposite effect in the other main neurogenic region of the brain, the subventricular zone, where neurosphere numbers decreased by approximately 40% in response to depolarizing levels of KCl. Most importantly, we also show that the latent hippocampal progenitor population can be activated in vivo in response to prolonged neural activity found in status epilepticus. This work provides the first direct evidence of a latent precursor and stem cell population in the adult hippocampus, which is able to be activated by neural activity. Since the latent population is also demonstrated to reside in the aged animal, defining the precise mechanisms that underlie its activation may provide a means to combat the cognitive deficits associated with a decline in neurogenesis.