Keio University

December 24, 2021
Keio University School of Medicine

Stimulation of Transplanted Human iPSC-derived Neural Cells Successfully Improves Therapeutic Effects in Spinal Cord Injury

Enhancing synaptic activity through selective stimulation of transplanted cells using synthetic receptor technology

A research team at Keio University School of Medicine has succeeded in transplanting human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) [1] into mice with spinal cord injury (SCI) to promote motor function recovery by stimulating the transplanted cells from outside the body using a synthetic receptor technology called DREADDs [2] to repeatedly increase the activity of the transplanted cells. The team was led by Professor Masaya Nakamura of the Department of Orthopedic Surgery, Professor Hideyuki Okano of the Department of Physiology, Instructor Momotaro Kawai, and Assistant Professor Narihito Nagoshi.

The research team has previously reported on the efficacy of transplanting hiPSC-NS/PCs into animal models of subacute SCI [3] to improve motor function. Here, the team conducted a detailed study that focused on the activity of hiPSC-NS/PCs following transplantation. They then introduced the DREADD gene and consecutively and selectively stimulated transplanted hM3Dq-NS/PCs in mouse models following subacute SCI. The results showed increased synaptic activity [4] and improved locomotor function in the stimulated animals when compared with the group of mice that had only undergone conventional transplantation.

The results of this study reveal the importance of hiPSC-NS/PCs activity and of synaptic activity between transplanted cells and the surrounding host tissue for SCI. These findings provide a strategy for developing therapies aimed at improving the efficacy of cell transplantation therapy for SCI.

The results of this research were published online on November 23, 2021 (EST), in the open-access journal *Cell Reports*.

1. Research Background

Spinal cord injury (SCI) can result from various circumstances, including traffic accidents, and patients can suffer irreversible damage to the motor, sensory, and autonomic nervous systems below the site of the injury. While there is still no effective clinical treatment for SCI, the total number of patients in Japan continues to increase and is currently estimated

to be between 100,000 and 200,000, and the treatment of subacute and chronic SCI patients are both considered to be major challenges.

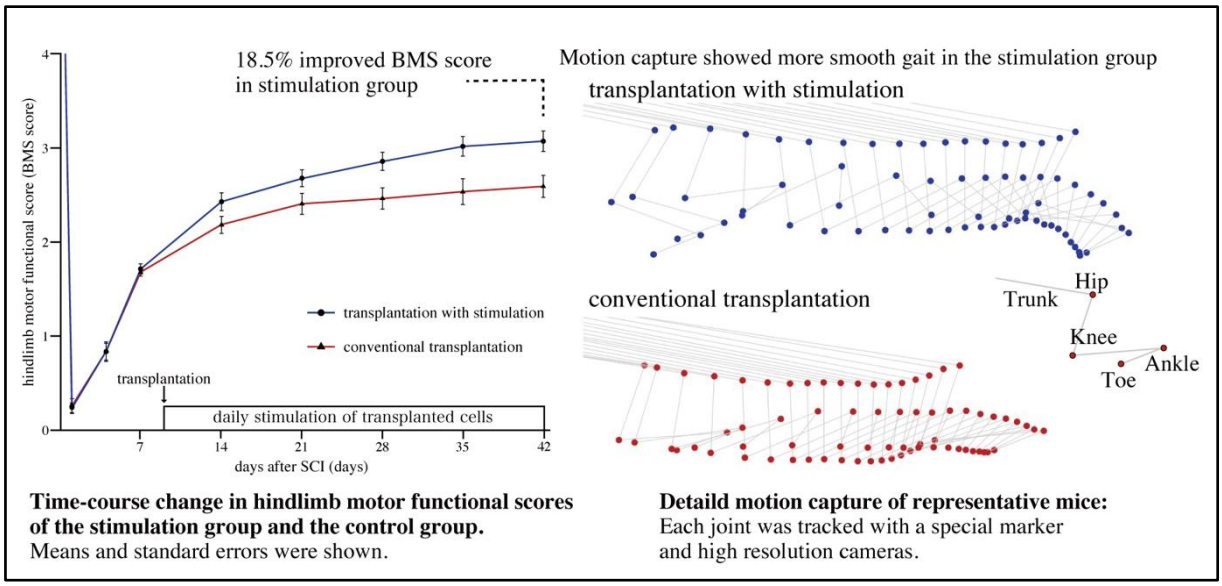
With the aim of developing regenerative treatments for SCI, the research team has completed the world's first transplantation of human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs) into rodent and primate models of SCI, successfully restoring locomotor function. Following this success, the team has continued their research toward the clinical application of regenerative therapy for SCI and has started the clinical trial "Regenerative medicine for spinal cord injury at subacute stage using human induced pluripotent stem cell-derived neural stem/progenitor cells" (UMIN ID: UMIN000035074, Regenerative Medicine Provision Plan ID: jRCTa031190228).

As part of their research to further improve the therapeutic potential of cell transplantation therapy, in this study, the team focused on investigating the activity of transplanted hiPSC-NS/PCs. In the developing nervous system, the activity of immature neurons has an important role in forming and maintaining synapses. Thus, the team investigated whether enhancing the activity of transplanted hiPSC-NS/PCs affected synaptic function and locomotor functional improvement.

To selectively stimulate transplanted cells, the team utilized newly developed synthetic receptors and verified the therapeutic effects of long-term selective stimulation of transplanted cells, compared to conventional cell transplantation therapy alone.

2. Research Significance and Future Development

The synthetic receptor DREADD gene was introduced into hiPSC-NS/PCs before transplantation. In mice with SCI, hiPSC-NS/PCs were transplanted in the subacute phase, followed by daily stimulation of the transplanted cells, and then compared with the group that had only undergone conventional cell transplantation. This resulted in improved locomotor function in the stimulated group when compared with the group that had only undergone conventional transplantation. (See figure below)



Time-course change in hindlimb motor functional scores of the stimulation group and the control group.
Means and standard errors were shown.

Detaild motion capture of representative mice:
Each joint was tracked with a special marker and high resolution cameras.

The transplanted cells were found to differentiate into neurons and form synapses with surrounding host cells, and stimulation was found to enhance the expression of synapse-related genes and proteins in surrounding host tissues. These findings provide a strategy

for developing therapies aimed at improving the efficacy of cell transplantation therapy for SCI.

3. Notes

This research was supported by the following programs:
- Research Center Network for Realization of Regenerative Medicine by the Japan Agency for Medical Research and Development (AMED)
- JSPS Grants-in-Aid for Scientific Research Program (KAKENHI) JP19K18541
- Keio University Doctoral Student Grant-in-Aid Program from the Ushioda Memorial Fund
- Keio University Grant-in-Aid for Encouragement of Young Medical Scientists
- AOSpine Research grant AOSJP(R)2019-02
- General Insurance Association of Japan Medical Research Grant

4. Research Paper

English Title: Long-term selective stimulation of transplanted neural stem/progenitor cells for spinal cord injury improves locomotor function
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Publication: *Cell Reports* (online)
DOI: 10.1016 / j.celrep.2021.110019

Glossary

[1] human induced pluripotent stem cell-derived neural stem/progenitor cells (hiPSC-NS/PCs)

hiPSC-NS/PCs have the ability to both self-renew and proliferate in an undifferentiated state and can differentiate into the three cell lineages that make up the central nervous system (neurons, astrocytes, and oligodendrocytes).

[2] DREADDs (Designer Receptor Exclusively Activated by Designer Drugs)

A DREADD (Designer Receptor Exclusively Activated by Designer Drugs) is a type of artificial engineered protein receptor, recently developed through the genetic modification of endogenous receptors. They respond exclusively to CNO (clozapine N-oxide), which has no pharmacological effect in humans and can be used to manipulate the activity of neurons. DREADDs have attracted attention for their ability to selectively manipulate target cells. In this study, the group used the DREADD hM3Dq, which can enhance the activity of transplanted cells.

[3] subacute SCI

The subacute period for SCI is considered to be approximately 9 days after injury in mice and 2–4 weeks in humans. The ideal time for cell transplantation is when the inflammatory response in the acute phase immediately following SCI has subsided, before scarring inhibits tissue regeneration.

[4] synaptic activity
Neurons are connected to each other at junctions called synapses, and information is transmitted between neurons via signals that pass through these synapses. Neurons with high synaptic activity are thought to be actively involved in the transmission of information in neural circuits.

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