Press Release

Keio University

Eisai

October 19, 2018

For Media and News Outlets

Keio University School of Medicine
Eisai Co., Ltd.

Discovery of Candidate Compound as Potential Treatment for Parkinson's Disease Using iPS Cells

—Opening the Way to Establishing New Techniques for Developing Treatments for Neurological Diseases—

A joint research group centered around Professor Hideyuki Okano and Associate Professor Jun Kohyama, Department of Physiology of the Keio University School of Medicine, together with a research group of Eisai Co., Ltd (CEO: Haruo Naito, "Eisai") has identified a compound that has the potential to be a treatment for Parkinson's disease by using dopaminergic neurons differentiated from induced pluripotent stem (iPS) cells from patients with familial Parkinson's disease.

Aiming to develop treatments for Parkinson's disease, this research group utilized neural progenitor cells induced from iPS cells derived from patients with familial Parkinson's disease and established a differentiation protocol for the stable supply of a large number of dopaminergic neurons. Furthermore, the research group screened an existing drug library as an indicator of susceptibility to stress observed in dopaminergic neurons derived from Parkinson's disease patients, and identified compounds that inhibit calcium channels. Further detailed analysis conducted by the research group revealed higher expression of T-type calcium channels in dopaminergic neurons derived from PARK2 patients. It was also found that apoptosis of dopaminergic neurons derived from Parkinson's disease patients could be reduced by inhibiting calcium influx via T-type calcium channels.

From these results, it is suggested that combining disease specific iPS cells and existing drug library has potential for both the development of treatments and clarification of disease pathology.

The results of this research was published in the online version of *Stem Cell Reports* at 12:00 noon on October 18, 2018 (EST).

1. Background and Outline of Research

Parkinson's disease is the second most common neurodegenerative disorder after

Alzheimer's disease, and causes motor symptoms such as tremor, bradykinesia, rigidity and postural instability and autonomic dysfunction due to a preferential loss of dopaminergic neurons in the substantia nigra. For 90% of Parkinson's disease patients, symptoms are idiopathic and it is difficult to understand the relationship between various factors, including those associated with the environment, and the disease mechanism. However, approximately 10% of patients show the familial incidence of Parkinson's disease, therefore understanding the disease mechanism for familial Parkinson's disease may also link with the understanding of Parkinson's disease including idiopathic Parkinson's disease as well as drug development.

Through the use of iPS cell technologies developed by Professor Shinya Yamanaka of Kyoto University in 2006, research has made great advances even in diseases that were difficult to be investigated by conventional methods. In 2012, the Keio University School of Medicine became the first research facility in Japan to create iPS cells from patients with familial Parkinson's disease, and was successful in replicating the disease mechanism. Currently research into neurological diseases has become very active throughout the world, and the understanding of diseases and development of new treatments is highly anticipated.

In April 2013, the Keio University School of Medicine and Eisai initiated the "Innovative Drug Discovery Project for Refractory Neurological Diseases Using iPS Cell Technologies," and since then this drug discovery project has been progressing as an industry-academia collaboration, making full use of Keio University School of Medicine's iPS cell and related technologies as well as Eisai's drug discovery techniques. Through this research, dopaminergic neurons which are thought to be damaged in Parkinson's disease patients are created efficiently and easily using iPS cells derived from Parkinson's disease patients, and an experimental system for conducting screening for drug discovery was established. In this research, Keio University School of Medicine's library of over 1,000 existing drugs was screened to find compounds preventing increased susceptibility to stress in dopaminergic neurons derived from Parkinson's disease patients.

2. Results and Significance of Research, Future Development

In this research, neural progenitor cells induced from iPS cells established from two familial Parkinson's disease (PARK2) patients were used to efficiently generate dopaminergic neurons. In patient-derived neurons, reduced neurite length as well as elevated oxidative stress and apoptosis were observed compared to neurons derived from healthy controls. Furthermore, it was revealed that these disease-relevant phenotypes were also observed in dopaminergic neurons derived from isogenic PARK2 null iPS cells obtained by genome editing.

In addition, since patient-derived dopaminergic neurons showed high susceptibility to rotenone, a mitochondrial respiratory chain complex I inhibitor, an existing drug library was screened with vulnerability to this drug-induced stress as the target. From phenotypic screening with an existing drug library, several compounds that suppressed stress-induced apoptosis were identified. Furthermore, it was found that T-type calcium channel

antagonists effectively reduced stress-induced apoptosis. Importantly, these compounds demonstrated similar reduction in stress-induced apoptosis in dopaminergic neurons derived from other type of familial Parkinson's disease (PARK6) patients who possess a genetic abnormality which is different to PARK2.

Further detailed analysis conducted by the research group revealed higher expression of T-type calcium channels in dopaminergic neurons derived from PARK2 patients. It was also found that apoptosis of dopaminergic neurons derived from Parkinson's disease patients could be reduced by inhibiting calcium influx via T-type calcium channels.

From these results, using iPS cells derived from patients enabled the establishment of *in vitro* disease models that reflect human biology, and by further combining with existing drug library, suggested efficacy for drug screening. By promoting the understanding of disease pathology through this method, it is hoped that this will lead to application in the development of a fundamental treatment for Parkinson's disease. Going forward, this research will continue, the knowledge gained will be further developed, and experimental systems closely reflecting the brain environment *in vivo* such as co-cultured neurons with glial cells will be utilized in an effort to verify the validity of targets for Parkinson's disease.

3. Special Notes

This research was supported by JSPS KAKENHI Grant Numbers JP16K15240, JP26713047, AMED under Grant Numbers JP17bk0104016h0005 and JP15bk0104009h0003, as well as joint research with Eisai Co., Ltd.

4. Academic Paper

Title: T-type calcium channels determine the vulnerability of dopaminergic neurons to mitochondrial stress in familial Parkinson's disease

Authors: Yoshikuni Tabata, Yoichi Imaizumi, Michiko Sugawara, Tomoko Noda (Ando), Satoe Banno, MuhChyi Chai, Takefumi Sone, Kazuto Yamazaki, Masashi Ito, Kappei Tsukahara, Hideyuki Saya, Nobutaka Hattori, Jun Kohyama, Hideyuki Okano

Publisher: *Stem Cell Reports*

[Glossary of Terms]

(1) iPS cell: Cells that are created from introducing specific transcription factors into somatic cells such as skin tissue, are self-proliferating and can be differentiated into all types of tissues and cells.

(2) Neural progenitor cells: Cells that can be differentiated into neurons or glia cells (astrocytes, oligodendrocytes).

(3) T-type calcium channels: A type of ion channels (proteins on the cell membrane) that selectively allows calcium ions to enter into cells from outside them.

(4) PARK2 (*parkin*): Familial Parkinson's disease which is more common in Japan. It is known that aberrations in PARK2 genes cause juvenile Parkinson's disease.

(5) PARK6 (*PINK1*): Autosomal recessive early-onset Parkinson's disease, the next most common type of Parkinson's disease after PARK2.

Research Inquiries

Professor Hideyuki Okano
Associate Professor Jun Kohyama
Department of Physiology
School of Medicine
Keio University
TEL : +81-3-5363-3747
FAX : +81-3-3357-5445
E-mail : hidokano@a2.keio.jp
E-mail : jkohyama@a7.keio.jp
http://www.okano-lab.com

Press Release Inquiries

Office of General Affairs
Shinanomachi Campus
Keio University
35 Shinanomachi, Shinjuku-ku
Tokyo 160-8582
TEL:+81-3-5363-3611
FAX:+81-3-5363-3612
E-mail: med-koho@adst.keio.ac.jp
http://www.med.keio.ac.jp/en/index.html

*A color version of this press release is available. Please contact the above address for more information.

Eisai Co., Ltd.
PR Department
TEL : +81-3-3817-5120

Eisai Co., Ltd.
PR Department
TEL : +81-3-3817-5120