── 招日研究助成報告 ──

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Dr. Chenは中国広州のJinan Universityの第一病院の小児科医で，てんかんの分子病態研究に興味を持ち，福岡大学てんかん分子病態研究所で研究に従事することになった。

Dr. Chenの今回の研究目的は，培養神経幹細胞に遺伝子編集技術であるCas9/CRISPRを用いて，てんかん脳症の遺伝子異常を導入し，神経細胞に分化させ，その病態を研究するものであった。対象疾患として，その80％程度の症例に遺伝子異常が見いだされるDravet症候群を選んだ。Dravet症候群では，Naチャネルの一つであるNav1. 1のα1サブユニットをコードする遺伝子SCN1Aに変異が見つかっている。このため，培養神経幹細胞にDravet症候群で発見されたSCN1A遺伝子の変異を導入することとした。

Dr. Chenは来日前には，基礎医学研究の経験がなかったため，細胞培養の基礎手法から学んでいただいた。慣れない作業ながら，Dr. Chenは努力を続け，細胞培養操作やその技術の習得をした。次に対象となる，Dravet症候群で発見されたSCN1A遺伝子に変異を導入するべく，最も効率よい遺伝子変異導入できる部分など，遺伝子情報を頼りに自らCas9/CRISPRに適した遺伝子変異を選択した。Dr. Chenは遺伝子に関する基礎研究の経験はなかったが，福岡大学に寄せられる多くの遺伝子検査用のサンプルから遺伝子変異をサンガー法を用いて解析するなどして，次第に遺伝学的知識の習得，また初歩的な遺伝子工学の実験手法などを学んだ。

今回の培養神経細胞に遺伝子変異を導入して，puromycinでのセレクションで，遺伝子変異が挿入されたとみられる株を採取することに成功した。現在福岡大学医学部小児科で，SCN1A遺伝子に変異が導入されている株をスクリーングしている。その後，目的外に遺伝子導入がないこと，染色体に異常がないことを確認して，今後のDravet症候群の分子病態研究に使用予定である。

Dr. Chenは明るく，聡明であった。また非常に優秀かつ勤勉で，私を始め研究室の各人に大きな影響を与えた。Dr. Chenは帰国後，中国広州のJinan Universityの小児科講師となり，現在も診療の傍らてんかんの分子生物学研究を継続している。

Chong-feng Chen, MD & PhD is a pediatrician at Department of Pediatrics of The First Affiliated Hospital of Jinan University in Guangzhou, China. She became interested in basic research for the molecular pathomechanisms of epilepsy. With this support from the foundation, she decided to conduct some basic experiment at Research Institute for the Molecular Pathomechanisms of Epilepsy Fukuoka University.

The research objective of her study at the institute was to introduce mutations, which have been identified in epileptic encephalopathy, to cultivated neuronal stem cells using a gene editing

method. The neuronal stem cells with gene mutation will be differentiated to neurons, which allows investigation on the pathomechanism of targeted epilepsy. In addition, such artificial patient neurons will be a good model in searching a new and effective treatment for epilepsy. To do this, she chose Dravet syndrome as a target disease. Dravet syndrome is the most devastating type of epileptic encephalopathy. Dravet syndrome is know to be caused by a number of mutations in *SCN1A*, the gene encoding the a1 subunit of neuronal voltage gated sodium channel, Nav1.1. The CRISPR/Cas9 system was employed to edit the genome of the neuronal stem cells. A few *SCN1A* mutations were selected according to the high probability of mutation introduction based upon the corresponding gene structures and relevant reagents have been prepared. The mutations were successfully introduced to the genome of the neuronal stem cells according to the conferred puromycin resistance. She has harvested several positive clones out of the cells survived after puromycin selection. Now, the colons are carefully investigate with regard to the correct introductions of the mutations followed by the quality control such as absence of extra target mutations and integrity of chromosome, etc. The cell she achieved should provide new insights on the molecular pathomechanisms of Dravet syndrome. Furthermore, the understanding of the pathomechanisms with the cellular models of Drave syndrome should contribute in finding new and effective treatments for Dravet syndrome.

Dr. Chong-feng Chen is bright and diligent, which always impressed all the members of the institute. She now retuned to Department of Pediatrics of The First Affiliated Hospital of Jinan University and working as assistant professor.

First of all, I would like to express my sincere appreciation to The Japan Epilepsy Research Foundation for giving me the excellent opportunity to study the way to pursue basic research for epilepsy. This wonderful experience in Japan would not have come true without the supports from the foundation. Though I had clinical interests in epilepsy before coming to Japan, I had not touched on the basic research on epilepsy. I needed to be used to not only all kinds of lab technique but also some specific related terms. However, the research project that I was assigned at the Research Institute for the Molecular Pathomechanisms of Epilepsy Fukuoka University very much interested and motivated me. The research goal was to establish artificial epilepsy patient neuronal stem cells. Nowadays, the genetic defects identified in genetic diseases can be introduced to live cells with the gene editing systems such as CRISPR/Cas9 technology. I have introduced several mutations of a gene called SCN1A. SCN1A mutations are know to cause Dravet syndrome, a type of intractable childhood epilepsy. In this project, there were many challenges in many respects. Whenever I faced such challenges, I was offered kind helps from many laboratory members at the institute. I really thank them for their kind supports give to me specifically when I did not speak Japanese very well. Though I could not completed the project because of the limited time, I believe that the cells I partially established in the projects will be used for future experiments for epilepsy whereby I will contribute to helping patients with Dravet syndrome. Besides the scientific experience I learnt in Japan, I has outstanding opportunity to learn Japanese culture, which impresses me very much. Last but least, I would like to thank Japan for widening my visons. Thank you all.