No.	K24-1114	
Project Title	Uncovering human neural differentiation by advanced multi-omics for the regeneration of brain function	
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	Project Member (s)	Shungo Adachi (National Cancer Center Research Institute / Department of Proteomics・Chief)

(Form 2)

Principal Investigator
Yasushi Saeki

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Annual Report

Report

Background and the research status

In this study, we aim to perform a comprehensive **multi-omics** analysis to profile transcription, translation, and proteolysis in neurons derived from human iPSCs at each stage of differentiation. Neural differentiation encompasses a heterogeneous population of cell types, including stem cells, progenitor cells, differentiated neurons, and glial cells. We utilized NGN2-TagRFP (expressed in neurogenic progenitor cells)/TUBB3-EGFP (expressed in post-mitotic neurons) dual-fluorescent reporter human iPSCs, which were established in our laboratory (*Park et al, 2022*), to monitor neural cell differentiation in living cells. In 2024, we conducted large-scale cell culture and FACS to generate comprehensive datasets profiles during human neural differentiation with collaborators: RNA-seq (One-stop sharing facility center at the Faculty of Pharmaceutical Sciences, Univ of Tokyo), ribo-seq (Shintaro Iwasaki (RIKEN), and proteomics (Shungo Adachi (National Cancer Center)).

Research progress in 2025 (2nd year)

Following sequencing and mass spectrometry analyses of all samples in the previous year, Gyeong-un Jang in my lab at KBRI (Daegu, Korea) conducted bioinformatics analyses to identify key molecules and pathways involved in human neural differentiation. We initially plotted the upregulated and downregulated molecules in newborn neurons and mature neurons using RNA-seq/proteomics data sets. Approximately 1,500 to 2,000 molecules exhibited non-linear alignment, identified as outliers. To investigate the mechanisms underlying such “RNA-protein decoupling”, we analyzed the role of upstream open reading frames (uORFs) during human neural differentiation. Multiple lines of evidence suggest that ribosomal stalling in uORFs can delay protein synthesis. Notably, the presence of proline clusters within uORFs amino acid sequences was significantly correlated with reduced protein production relative to RNA level. Furthermore, ribosome profiling (ribo-seq) peak analysis supports the hypothesis that proline-enriched domains can delay protein synthesis in specific molecules.

These findings were presented at the IMSUT collaborative project’s internal online symposium in February 2026. In the 3rd year of the research period, we aim to elucidate the molecular mechanisms underlying uORF-mediated regulation of protein synthesis and to assess their significance in human neural differentiation using multiple approaches, including genome editing and brain organoid experiments.