News Release

Title

Unveiling synapse pathology in spinal bulbar muscular atrophy by genome-wide transcriptome analysis of purified motor neurons derived from disease specific iPSCs

Key Points

- *Disease specific iPSCs from SBMA patients were generated and differentiated into motor neurons, followed by flow cytometry and cell sorting to purify motor neurons.*
- *Comprehensive transcriptome analysis showed enrichment of genes regarding synapse, neurotransmitters, exocytosis, and epigenetics in SBMA motor neurons.*
- *Upregulation of RSPO2 and WNT ligands was indicated in SBMA motor neurons, both of which are crucial for synapse formation.*
- *Based on these results, elucidation of SBMA pathophysiology and identification of novel therapeutic targets focusing on neuro-muscular pathology is expected.*

Summary

Pathological analysis of spinal bulbar muscular atrophy (SBMA) is being forward by the team led by Assoc Prof Yohei Okada and Researcher Kazunari Onodera (Nagoya University Graduate School of Medicine, Visiting Researcher of Neurology) at Aichi Medical University School of Medicine, Department of Neurology, and collaborators including Keio University School of Medicine, Nagoya University Graduate School of Medicine, Niigata University Graduate School of Medical, and Tokyo Medical and Dental University/the University of Tokyo. They established a new SBMA disease model by generating iPSCs from SBMA patients and differentiated them into motor neurons (MNs). By comprehensive transcriptome analysis, they found that synapses are crucially associated with neuronal degeneration, which rises expectation for elucidation of SBMA pathophysiology and therapeutic development. The results of this research were published online in the British science journal, Molecular Brain on February 19, 2020.

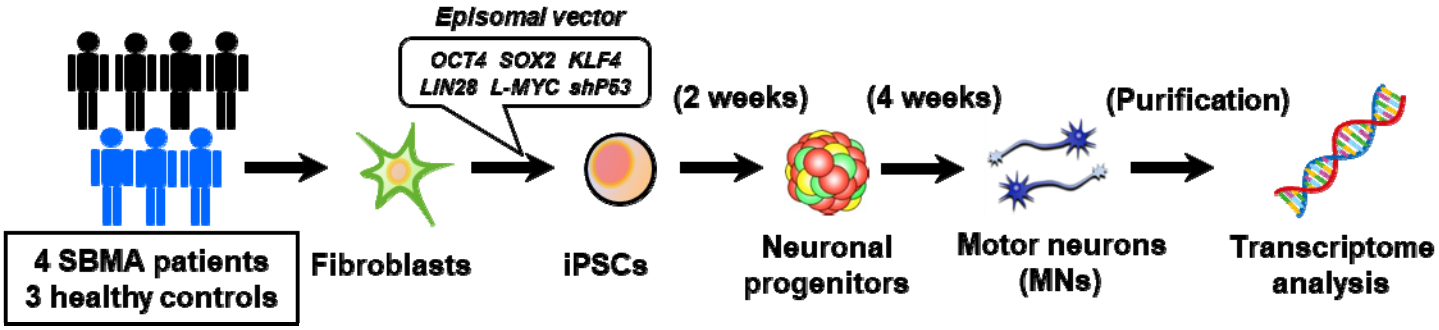

Fig. 1. Pathological analysis using SBMA patients-derived iPSCs

Research Background

SBMA is an adult-onset slowly progressive lower MN disease caused by abnormal CAG repeat expansion in the androgen receptor (AR) gene. SBMA is characterized by weakness and atrophy of limbs and bulbar muscles caused by the degeneration of spinal and bulbar MNs. Through analysis using transgenic mice models harboring mutant AR with expanded polyglutamine tract (AR-97Q) and *in vitro* cell culture models, mutant AR has been shown to form nuclear aggregation in a ligand (testosterone)-dependent manner, causing MN degeneration. However, mechanisms underlying neuronal degeneration in SBMA are not yet fully elucidated, as SBMA model mice exhibit several discrepancies with human SBMA patients. Therefore, a novel human disease model that more accurately recapitulates SBMA patients' pathology has been expected for more precise pathophysiological analysis and the development of novel therapeutics.

It is usually difficult to collect human-derived MNs alive. Therefore, this research group provided a new SBMA disease model by establishing iPSCs from SBMA patients and differentiating them into MNs, followed by the analysis of disease pathology using SBMA patients-derived MNs.

Research Results

The research group first generated iPSCs from skin fibroblasts of four SBMA patients. The number of CAG repeat in the causative AR gene did not change during the process of iPSC establishment (reprogramming). The iPSCs were differentiated into MNs and cultured for maturation for 4 weeks. In order to increase the purity of the MNs to be analyzed, MNs were specifically labeled with a fluorescent reporter (*HB9*^{e438}*::Venus*) and fractionated by flow cytometry and cell sorting. The enriched MNs thus showed reduced heterogeneity and uniform MN induction efficiency among iPSC clones. Total mRNA was extracted from the purified MNs, followed by RNA sequence analysis to investigate genes showing altered expression in SBMA compared with healthy controls (Fig. 1).

In gene ontology and pathway analysis, genes of synapses-, neurotransmitters-, exocytosis-, and epigenetics-related categories were highly expressed in SBMA MNs. In addition, decreased expression of genes in the ER category was observed (Fig. 2).

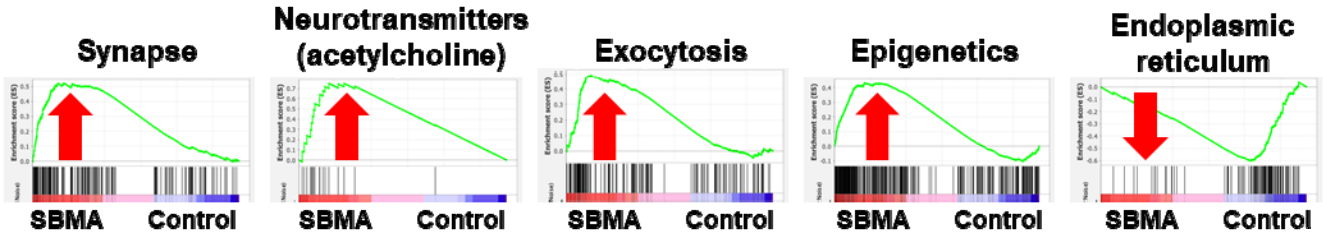

Fig. 2. Enrichment plots: Gene categories enriched in SBMA MNs

What should be noted, synapse-related genes included increased expression of *RSPO2* gene and WNT ligands, which promote clustering of acetylcholine receptors (AChRs) essential for the formation of the neuromuscular junctions (NMJs) (Fig. 3). Furthermore, increased expression of the *WNT3A* gene, which negatively regulates NMJ formation, was observed. These results suggest that they may be responsible for the dysregulation of neuromuscular synapses.

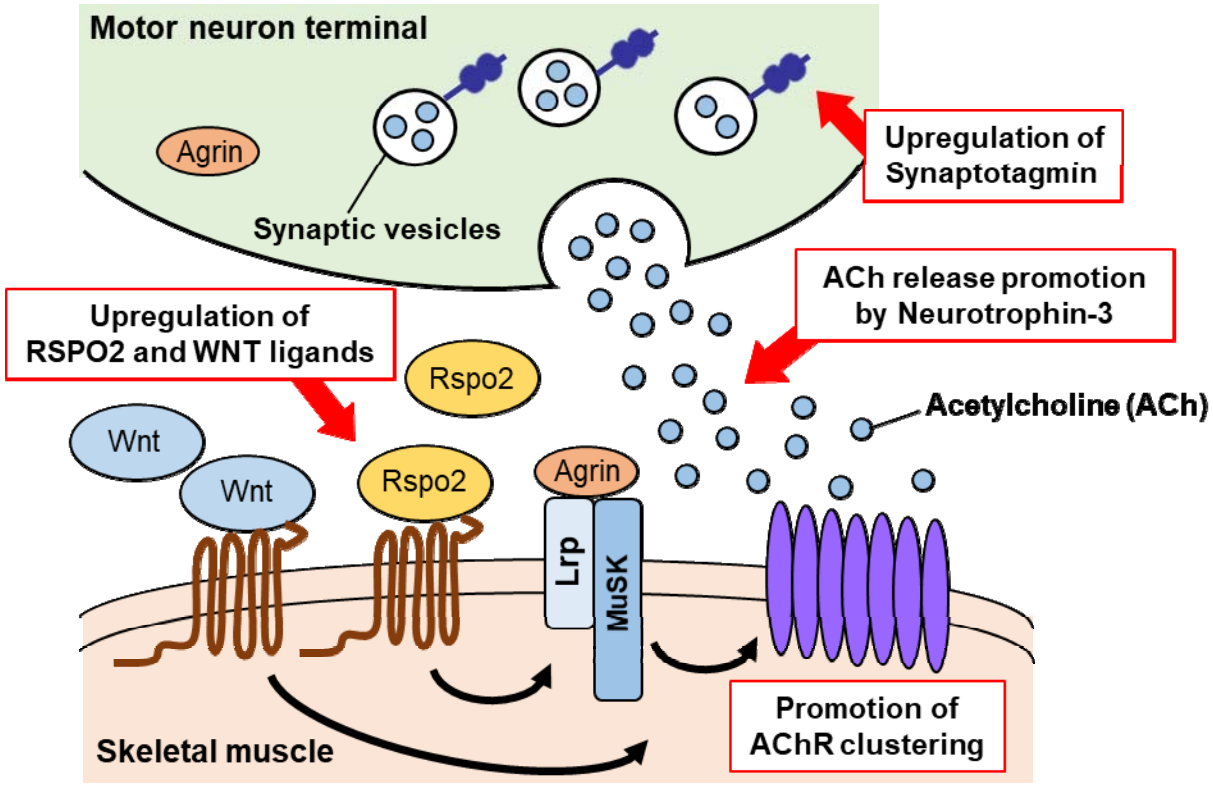

Fig. 3. Gene expression alterations responsible for NMJ formation

Research Summary and Future Perspective

The results of this study have revealed that synaptic pathology is deeply involved in MN degeneration in SBMA. Since the degeneration of NMJ has been shown in mice models, aberrant expression of synapse-related genes in SBMA iPSC-derived MNs was shown for the first time. Such gene alterations will provide a clue to understand the pathology of SBMA, and the development of NMJ-targeted therapeutics to suppress the disease progression is expected.

Publication

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