FOXG1 Syndrome Using hiPSC-derived Cortical Organoids

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Background

FOXG1 syndrome is a rare and debilitating childhood neurological disorder caused by a mutation in the FOXG1 gene. FOXG1 (14q22.1) is a one exon gene that encodes for forkhead box protein G1 (FOXG1), a transcriptional repressor involved in orchestrating embryonic brain development; in particular, the development of telencephalon (1-4). FOXG1 was found responsible for maintenance and proliferation of neuronal stem cell progenitors (5-7), neuronal migration (8), callosal axon guidance (9), to name a few. FOXG1 has a complex mechanism of action as it was found to regulate cellular expression via both DNA-binding-dependent and independent pathways (10). Dysregulation of FOXG1 expression results in symptoms that include microcephaly, severe intellectual disability, epilepsy, stereotypies, dyskinesias, corpus callosum agenesis and other associated conditions (11).

The onset of FOXG1 syndrome happens during early stages of embryogenesis; hence, cortical organoid model, which was found to replicate early stage brain expression programs and neuronal network formation (12), is a promising platform to probe FOXG1 mechanisms. Considering FOXG1 syndrome symptoms, we hypothesize that patient-derived organoids would show decrease in circumference and neuronal rosette formation as well as deviations in electrophysiological studies. Inasmuch, organoid model has potential to uncover previously unknown human-specific pathways.

Aims and Goals

Aim 1. Generate and characterize patient derived cortical organoids to compare in vitro and in vivo phenotypes; and to study FOXG1 mechanisms and downstream binding partners.

Aim 2. Based on the outcome of aim 1, propose and design potential therapeutic solutions with hopes of rescuing the phenotypes associated with FOXG1 syndrome in cortical organoids.

Materials and Methods

Patient fibroblasts were Sendai reprogrammed to a pluripotent state. Generated iPSCs were validated by immunohistochemistry (IHC) with pluripotency markers such as SOX2, OCT4, and NANOG. Patient-derived iPSCs were then collected to generate brain organoids from the expression of pluripotency markers such as SOX2, OCT4, and NANOG. Patient-derived iPSCs were used. After organoids reached 1 month of age, they were analyzed by IHC, microelectrode array (MEA) and mycoplasma free iPSCs were used. After organoids reached 1 month of age, they were analyzed by IHC, microelectrode array (MEA) and Western Blot (WB). To ensure the efficacy of the detection assays, CRISPR/Cas9 lines were generated for FOXG1 heterozygous knock-out (FOXG1+/-) and FOXG1 homozygous knock-out (FOXG1–/–) which will be compared with FOXG1 isogenic control (FOXG1 WT). Organoids were generated for CRISPR-derived lines. FOXG1+/– and FOXG1–/– were used to evaluate specificity of anti-FOXG1 antibody.

Summary and Conclusions

• Skin cells from 6 patients carrying FOXG1 genetic variants were successfully reprogrammed via Sendai virus.
• iPSC express pluripotency markers and are karyotypically normal.
• Cortical organoids were generated from all patient-derived hiPSC lines and CRISPR/Cas9 genome edited FOXG1+/– and FOXG1–/–.
• Cortical organoids from p.Gln326*+129 line exhibit lower neuronal activity compared to controls. Presented data serves as a proof of concept for generation of FOXG1 syndrome organoids as validated by IHC. Initial findings support our hypothesis about cortical organoid model and FOXG1 syndrome.

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