Modeling FOXG1 Syndrome Using hiPSC-derived Cortical Organoids

Veronika Mikhaylova, Wirla Pontes[1], Indrani Ganguly[1], Angels Almenar-Queralt[1], Alysson Muotri[1]
[1] UCSD at Sanford Consortium for Regenerative Medicine, La Jolla CA

Background

FOXG1 syndrome is a rare and debilitating childhood neurological disorder caused by a mutation in the *FOXG1* gene. *FOXG1* (14q12) 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[3,4].
FOXG1 was found responsible for maintenance and proliferation of neuronal stem cell progenitors[1,4], neuronal migration[1,5], callosal axon guidance[1], 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[6]. Dysregulation of FOXG1 expression results in symptoms that include microcephaly, severe intellectual disability, epilepsy, stereotypies, dyskinesias, corpus callosum agenesis and other associated conditions[3,8].
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[9] and neuronal network formation[7], 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 according to previously published protocol[7]. Only karyotypically normal 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.

Ground work

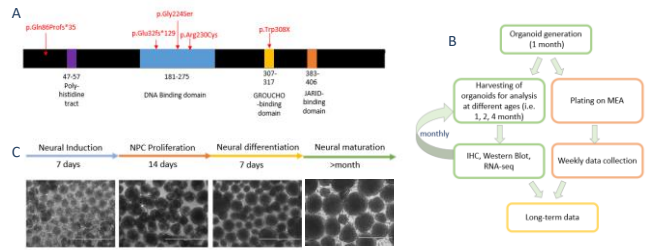

Figure 1. A) Schematic of FOXG1 protein, B) General project workflow C) Organoid generation schedule

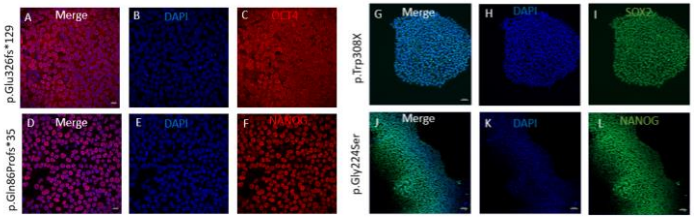

Figure 2. Immunohistochemistry on patient derived iPSCs with pluripotency markers A-C) OCT4, D-F and J-L) NANOG, G-I) SOX2. The images were take on Zeiss z-stack apotome at 40x magnification (A-F) and 20x magnification (G-L).

Summary and Conclusions

- Skin cells from 6 patients carrying FOXG1 genetic variants were successfully reprogrammed via Sendai virus.
- hiPSC express pluripotency markers and are karyotypically normal.
- Cortical organoids were generated from all patient-derived hiPSCs lines and CRISPR/cas9 genome edited FOXG1+/- and FOXG1-/-.
- Cortical organoids from p.Gln326fs*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.

Acknowledgements

Thank you to all graduate students, postdoctoral fellows, staff scientists and technicians at Dr. Muotri Lab.
Thank you to SDSU BSCRIP coordinators Dr. Feuer and Dr. Mokili.
Thank you FOXG1 Foundation.

Results

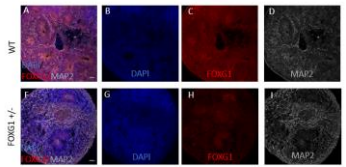

Figure 3. Immunohistochemistry analysis of FOXG1 antibody binding for A-D) Male control organoids and CRISPR/Cas9 generated lines F-I) FOXG1 +/- and K-N) FOXG1 -/-organoids

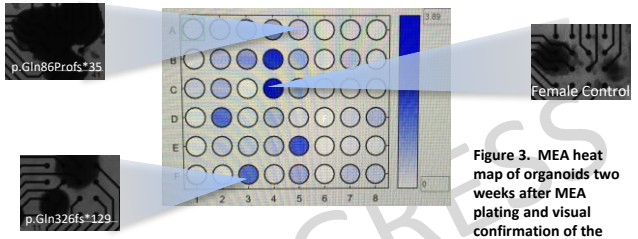

Figure 3. MEA heat map of organoids two weeks after MEA plating and visual confirmation of the organoid attachment

Future Directions

Short-term: To accomplish Aim 1, data collection (e.g. IHC, WB) will continue for FOXG1 cortical organoids at various time points such as 2, 4, 6, 8 month etc. Weekly MEA recordings throughout the lifespan of the organoids would provide sufficient data to analyze electrophysiological parameters.
Long-term: To accomplish Aim 2, potential therapeutics will be designed and verified on FOXG1 organoids. For instance, antisense oligonucleotide (ASO) therapies. ASO therapies showed promising results in patients with neurological conditions such as amyotrophic lateral sclerosis, Huntington's and Alzheimer's diseases[10]. ASO are designed to alter cellular expression by interacting with RNA via Watson-Crick interactions[10]. Thus, ASO therapy has potential to help FOXG1 syndrome patients with heterozygous *FOXG1* mutation by increasing expression of functional *FOXG1* copy.

References Cited

1.Cargnin, F. *et al.* FOXG1 Orchestrates Neocortical Organization and Cortico-Cortical Connections. *Neuron* **100**, 1083-1096.e5 (2018).
2.Mitter, D. *et al.* FOXG1 syndrome: Genotype-phenotype association in 83 patients with FOXG1 variants. *Genet. Med.* **20**, 98–108 (2018).
3.Ariani, F. *et al.* FOXG1 Is Responsible for the Congenital Variant of Rett Syndrome. *Am. J. Hum. Genet.* **83**, 89–93 (2008).
4.Zhu, W. *et al.* Precisely controlling endogenous protein dosage in hPSCs and derivatives to model FOXG1 syndrome. *Nat. Commun.* **10**, (2019).
5.Croci, S. *et al.* AAV-mediated FOXG1 gene editing in human Rett primary cells. *Eur. J. Hum. Genet.* (2020) doi:10.1038/s41431-020-0652-6.
6.Hettige, N. C. & Ernst, C. FOXG1 Dose in Brain Development. *Front. Pediatr.* **7**, 1–12 (2019).
7. Trujillo, C. A. & Muotri, A. R. Brain Organoids and the Study of Neurodevelopment. *Trends Mol. Med.* **24**, 982–990 (2018).
8. Vegas, N. et al. Delineating FOXG1 syndrome: From congenital microcephaly to hyperkinetic encephalopathy. Neurol. Genet. 4, (2018).
9. Camp, J. G. et al. Human cerebral organoids recapitulate gene expression programs of fetal neocortex development. Proc. Natl. Acad. Sci. U. S. A. 112, 15672–15677 (2015).
10. Bennett Frank, C., Krainer, A. R. & Cleveland, D. W. Antisense Oligonucleotide Therapies for Neurodegenerative Diseases. Annu. Rev. Neurosci. 42, 385–406 (2019).