Combining CRISPR-Cas9 editing with human cortical organoid model to explore the roles of FOXP1 in brain diseases

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Abstract:

FOXP1 is a transcription factor crucial for the functionality of the human brain from the first stages of development. Indeed, heterozygous mutations in this gene lead to the FOXP1 syndrome, a rare neurodevelopmental disorder clinically characterized by language impairment, intellectual disabilities and autistic traits. Yet, mouse models of FOXP1 haploinsufficiency or deficiency have failed to reveal major cortical phenotypes, suggesting human-specific pathomechanisms.

To gain insights into FOXP1 functions in a human context, we are leveraging human pluripotent stem cells (hPSCs) -derived cortical organoids (hCOs), 3D *in vitro* models able to recapitulate the main steps of human brain development, to characterize *FOXP1* spatiotemporal expression at the mRNA and protein levels. To further investigate its functions, we are using a CRISPR-Cas9 approach to knock-out (KO) *FOXP1* in hPSC lines that can be further used for hCO generation.

Our results show that in hCOs FOXP1 is expressed in neural progenitor cells in early stages, while at later stages it is downregulated in these cells, but becomes highly expresed in excitatory cortical neurons. Moreover, our study reveals the presence of different *FOXP1* isoforms in hPSCs and during hCO differentiation, highlighting the complex regulation of this *locus* during human brain development. We identify the canonical long isoform as the most abundant expressed in hCOs, while the ES isoform is prominent in hPSCs. Moreover, hCOs express shorter isoforms (albeit at lower levels), possibly resulting from an alternative transcriptional starting site (TSS). Finally, we show that our CRISPR-cas9 strategy targeting the first exons of FOXP1 *locus* through a plasmid-based CRISPR-Cas9 approach led to the generation of clonal hPSC lines with long isoform-specific KO that retain pluripotency and capability to generate hCOs.

These new models will allow us to further understand the role of FOXP1 variants during brain development and link the complex regulation of this locus to human brain dysfunctions.

In conclusion, our study underscores the utility of hPSC and hCO technologies for functional studies of human brain development and disease and calls for more attention to the understudied isoform diversity in brain pathologies.