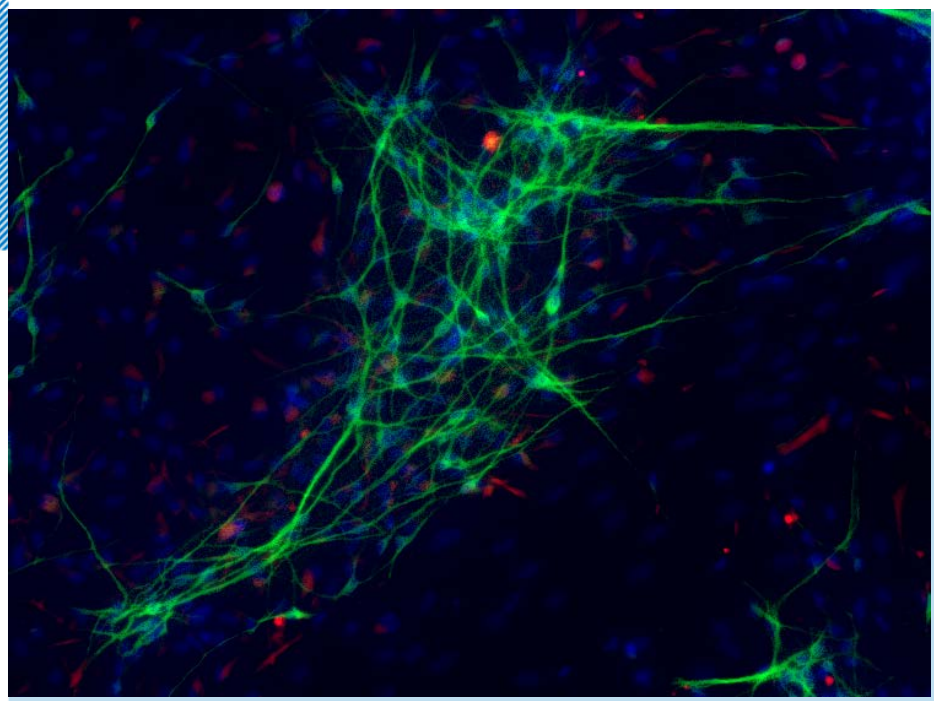
Leids Universitair Medisch Centrum

Molecular mechanisms and gene targeting therapies for the Foxp1 syndrome



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Inhoudsopgave

1. Nederlandse samenvatting

Mutaties in het FOXP1 gen veroorzaken verschillende syndromen die invloed hebben op de de neurologische ontwikkeling wat kan leiden tot verstandelijke beperking, spraak/taal achterstand of autistische eigenschappen. Er zijn 7 mutaties die in meer dan 1 persoon terug gevonden zijn en 5 van deze varianten liggen in het stuk van het FOXP1 eiwit dat aan het DNA kan binden. Dit is een belangrijke domein in het eiwit omdat door deze binding aan het DNA, het FOXP1 eiwit de expressie van andere eiwitten kan regelen.
In dit project willen we gaan onderzoeken hoe een mutatie in het DNA bindende domein van FOXP1 de neurologische ontwikkeling kan beïnvloeden. Dit doen we door middel van 'Brain-on-a-Chip' technologie waarmee het mogelijk is 3D hersenorganoids te maken. Door organoids te maken met en zonder de *FOXP1* mutatie, kunnen we onderzoeken waar en wanneer er veranderingen optreden in de ontwikkeling van de hersenen. Dit cel model zal belangrijk zijn voor verder studies naar het ontstaan van het FOXP1 syndroom en voor de ontwikkeling van therapieën. Ook zullen er verkennende studies gedaan worden om de fout in het DNA die het FOXP1 syndroom veroorzaakt, te verwijden met zogeheten Adenine Base Editors. Deze Base Editors zijn een variant op de genoom editing techniek CRISPR/Cas.

2. Research Project

2.1 Introduction

Haploinsufficiency of the forkhead-box protein P1 (*FOXP1*) gene causes a broad neurodevelopmental syndrome involving global developmental delay, intellectual disability (ID), speech/language impairment, and autistic features (MIM# 613670) [1]. A recent paper on the mutational landscape of FOXP1 described 34 private mutations and a further 7 mutations that were present in unrelated individuals [2]. Of the 7 recurring mutations, five were located in the DNA binding domain and one in the Zinc Finger domain of the FOXP1 protein.
One variant located in the DNA binding domain is an arginine-to-histidine substitution in (p.R514H) that was identified as an identical heterozygous *de novo* variant in three unrelated probands. This FOXP1 variant is equivalent to a variant previously identified in FOXP2 [3]. The FOXP1 protein can heterodimerize via its leucine zipper domain, while the **DNA-binding domain** can make direct contact with the back-bone of the target DNA where it can act as a transcription factor. The FOXP1 protein can regulate transcription in brain regions where FOXP1 and FOXP2 are co-expressed, such as the striatum and certain cortical neurons [4, 5], where they may cooperatively regulate downstream targets [6]. A dominant-negative effect of the an arginine-to-histidine substitution in (p.R514H) has been suggested, whereby the FOXP1 variant prevents the wild type FOXP1 protein from binding to DNA and regulating transcription. Brain-specific deletion of FOXP1 in mice impaired neuronal development and and cognitive and social deficits [7].

Studying human brain development and the pathobiology of brain disorders has traditionally been hampered by appropriate cell culture models. The possibility of generating patient-derived IPSC lines followed by differentiation into desired cell types can provide new insights into disease mechanisms and can facilitate translation of basic brain research into clinical application. Especially the application of **human iPSC-derived, 3D brain organoids** [8], that reflect many features of complex human brain development is promising for the study of FOXP1 protein function and the pathological consequences of the mutation in the DNA binding domain of FOXP1.

As a possible **treatment** that will target the specific gene variant (c.1541G>A(p.Arg514His)) in FOXP1, we will explore the use of **Adenine Base Editors** (ABEs) that can permanently convert A•T base pairs into G•C. This base editing is a form of genome editing that enables direct, irreversible conversion of one base pair to another at a target genomic locus without requiring double-stranded DNA breaks, homology-directed repair processes, or donor DNA templates [9]. Compared with standard genome editing methods to introduce point mutations, base editing can proceed more efficiently and with far fewer undesired products.

2.2 Research Questions

- What is the role of FOXP1 protein and the *FOXP1* p.R514H variant during early brain development
- Can DNA targeting therapies correct the G to A substitution that causes Foxp1 syndrome.

2.3 Aims of this project

1. To create induced pluripotent stem cell lines carrying the FOXP1 p.R514H variant and a FOXP1knock out cell line using CRISPR/Cas9.
2. Determine the role of FOXP1 protein during brain development using cerebral organoids from induced pluripotent stem cells.
3. Study the disease mechanism of the Foxp1 p.R514H variant in early brain development using cerebral organoids from induced pluripotent stem cells (See Figure 1).
4. Explore the potential of programmable adenine base editors (ABEs) to correct the G to A conversion in FOXP1 in genomic DNA.

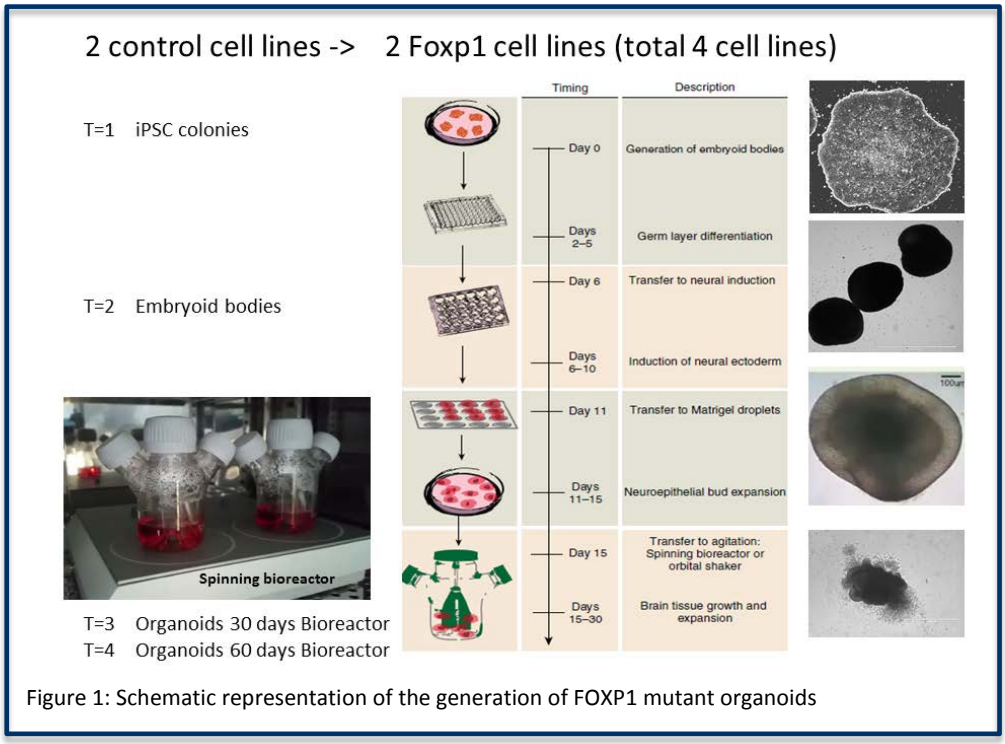

Figure 1: Schematic representation of the generation of FOXP1 mutant organoids

3. Time line and Budget

3.1 Time line

Year 1: (i) Generate isogenic FOXP1 iPS cell lines and neuroblastoma cell lines harboring the (c.1541G>A(p.Arg514His)) variant as well as a knock out version of *FOXP1*

(ii) Design programmable adenine base editors specifically recognizing the (c.1541G>A(p.Arg514His)) variant in *FOXP1*

Year2: (iii) Perform immunohistochemical studies and single cell RNAseq (scRNAseq) on iPSC, embryoid bodies, 30day old organoids and 60 day old organoids from control and Foxp1 mutant iPSC to study neurodevelopmental and gene expression changes.

(iv) Test programmable adenine base editors specifically recognizing the (c.1541G>A(p.Arg514His)) variant in *FOXP1* in a human neuroblastoma cell line

	Year 1				**Year 2**			
Tasks	Q1	Q2	Q3	Q4	Q5	Q6	Q7	Q8
CRISPR/Cas cell line generation								
iPSC organoid culturing								
Immuno organoids								
RNAseq organoids								
Design ABEs								
Test ABEs in human cells								

3.2 Budget

	Year 1	Year 2	Total
PostDoc salary (1.0 fte)			
Technician (0.2 fte)			
iPS cell culture			
scRNAseq			
Lab consumables			
Total cost			**€245.000**

4. Literature

1. Sollis, E., et al., *Identification and functional characterization of de novo FOXP1 variants provides novel insights into the etiology of neurodevelopmental disorder.* Hum Mol Genet, 2016. **25**(3): p. 546-57.
2. Siper, P.M., et al., *Prospective investigation of FOXP1 syndrome.* Mol Autism, 2017. **8**: p. 57.
3. Sollis, E., et al., *Equivalent missense variant in the FOXP2 and FOXP1 transcription factors causes distinct neurodevelopmental disorders.* Hum Mutat, 2017. **38**(11): p. 1542-1554.
4. Ferland, R.J., et al., *Characterization of Foxp2 and Foxp1 mRNA and protein in the developing and mature brain.* J Comp Neurol, 2003. **460**(2): p. 266-79.
5. Hisaoka, T., et al., *The forkhead transcription factors, Foxp1 and Foxp2, identify different subpopulations of projection neurons in the mouse cerebral cortex.* Neuroscience, 2010. **166**(2): p. 551-63.
6. Sin, C., H. Li, and D.A. Crawford, *Transcriptional regulation by FOXP1, FOXP2, and FOXP4 dimerization.* J Mol Neurosci, 2015. **55**(2): p. 437-48.
7. Bacon, C., et al., *Brain-specific Foxp1 deletion impairs neuronal development and causes autistic-like behaviour.* Mol Psychiatry, 2015. **20**(5): p. 632-9.
8. Lancaster, M.A., et al., *Guided self-organization and cortical plate formation in human brain organoids.* Nat Biotechnol, 2017. **35**(7): p. 659-666.
9. Gaudelli, N.M., et al., *Programmable base editing of A*T to G*C in genomic DNA without DNA cleavage.* Nature, 2017. **551**(7681): p. 464-471.