CRISPR-Cas9-based functional investigation of the “dark genome” in search of putative downstream effectors of Sox2 in neurodevelopmental disease

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Keywords: Sox2, T-dark genes, neurodevelopment, neurodevelopmental disease, brain organoids

Abstract:
Mutation of the Sox2 gene causes defective development of multiple brain regions, leading to blindness, mental retardation, seizures. SOX2, a transcription factor, controls the activity of many genes, some of which are known to be involved, if mutated, in other neurodevelopmental defects.We identified, by RNAseq, over thousand genes downregulated following Sox2 deletion in neural stem cells derived from the mouse developing brain. Many human genes are homologous to the mouse genes we found. Many of them belong to the T-dark gene category, genes of which very little is known. We chose 122 human T-dark genes, prioritizing those whose activity is more impaired in Sox2-deleted mouse neural cells. To identify those genes involved in neurodevelopment, we will use a screening procedure based on growing in vitro human brain “organoids” derived from a previously established pluripotent cell line. The screening is based on in vitro mutagenesis via the CRISPR-Lineage Tracing at Cellular resolution in Heterogeneous Tissue (LICHT) methodology: this technique is a combination of CRISPR-Cas9 mutagenesis technology and a dual barcode method, allowing to retrospectively identify the genes that have been mutated in underrepresented cell clones within brain organoids by genomic sequencing. We hypothesize that these T-dark genes may include important functional effectors of SOX2, whose downregulation may contribute to the abnormal phenotypes observed in mouse Sox2 mutants. The identification of neurodevelopmental functions for at least some of the investigated Sox2-regulated T-dark genes, should shed light into a deeper understanding of the Sox2-dependent regulatory program both in Sox2 pathology and in other neurodevelopmental diseases (NDD).
In conclusion, our project should identify neurodevelopmental functions for at least some of the investigated T-dark genes, paving the way for understanding their role in NDD, with the potential to generate new ideas towards therapeutic approaches.