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A CRISPR-Cas9-based high-throughput screen in human brain organoids of T-dark putative downstream effectors of SOX2 in neurodevelopmental disease

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Abstract:

Mutation of the SOX2 transcription factor gene causes a multi-facet neurodevelopmental disorder (NDD) in man and mouse. SOX2- dependent defects involve impaired neural stem/progenitor cell (NSC) proliferation and impaired differentiation/migration of forebrain GABAergic interneurons.(1). We identified, by RNAseq, over a thousand genes downregulated following Sox2 deletion in mouse neural stem cells (NSC)(undifferentiated, and differentiating towards GABAergic neurons and glia). Among these genes are many “T-dark” genes (of still unknown function; see https://pharos.nih.gov/), conserved between mouse and humans (2). We hypothesize that these T-dark genes may include important functional effectors of SOX2. We propose to investigate the neurodevelopmental function of SOX2-regulated T-dark genes, by a high-throughput targeted mutagenesis screen via CRISPR-Cas9, in differentiating three-dimensional (3D) human brain organoids (dorsal or ventral forebrain, the source of GABAergic interneurons in development), by differentiation of human pluripotent stem cells (3,4). We will use a recently developed high-throughput screening procedure, CRISPR-lineage tracing at cellular resolution (CRISPR-LICHT, ref. 3), generating targeted loss of function mutations in many genes. We will target loss of function mutations to 129 T-dark genes we identified among the human homologs of mouse genes, that are the most downregulated following Sox2 deletion in NSC. We will look for effects on NSC/progenitors proliferation and neuronal survival/migration. For the genes showing the most important effects in the primary screen, the subsequent development and characterization of single-gene mutant organoids will allow a more detailed understanding of their function. 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.

1-Mercurio S. et al (2019) More than just Stem Cells: Functional Roles of the Transcription Factor Sox2 in Differentiated Glia and Neurons. Int J Mol Sci 20. DOI: 10.3390/ijms20184540

2-Bertolini et al (2019), Mapping the Global Chromatin Connectivity Network for Sox2 Function in Neural Stem Cell Maintenance. Cell Stem Cell 24, 462-476 e466 DOI: 10.1016/j.stem.2019.02.004

3-Esk C.et al (2020) A human tissue screen identifies a regulator of ER secretion as a brain-size determinant. Science 370, 935-941. DOI: 10.1126/science.abb5390

4-Kim J. et al (2020) Human organoids: model systems for human biology and medicine. Nature reviews. Molecular cell biology 21, 571-584. DOI: 10.1038/s41580-020-0259-3