Dissection of the SOX2 and NR2F1 gene regulatory network in the developing visual thalamus

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Keywords: SOX2, NR2F1, visual system, zebrafish, mouse

Abstract:

SOX2 is a transcription factor well known for its role in neural stem cell maintenance and its mutations in humans lead to a neurodevelopmental disease characterized by severe visual defects. We have shown that SOX2 is also expressed in differentiated neurons of the dorsolateral geniculate nucleus (dLGN), a thalamic relay nucleus crucial for visual information processing. The dLGN receives visual input from retinal ganglion cells and projects to the primary visual cortex. Interestingly, SOX2 is co-expressed in dLGN neurons with the transcription factor NR2F1, whose mutations are also associated with human visual disorders. Conditional knockout (cKO) of *Sox2* and *Nr2f1* in thalamic neurons in mice leads to defective dLGN differentiation and visual system formation.

To define the gene regulatory network downstream of SOX2 in thalamic neurons, we performed RNA sequencing and CUT&RUN analyses on neonatal dLGNs from *Sox2* cKO mice. These experiments identified hundreds of reproducible SOX2 binding sites and thousands of differentially expressed genes, significantly enriched for functional categories related to neuronal differentiation, axon guidance, and synapse formation. About 500 of the deregulated genes in *Sox2* cKO where also deregulated in *Nr2f1* thalamic cKO.

We have characterized by *in situ* hybridization on mouse brain section the expression of potential common targets of these two transcription factors. We are studying the role of selected targets in zebrafish by generating F0 biallelic CRISPR-Cas9 knockouts in the Tg(atoh7:GAP-RFP) line, in which retina-optic tectum projections express RFP, to determine how they affect visual system formation. In parallel, we are setting up primary cultures of murine thalamic neurons to assess the function of the key SOX2 and NR2F1 targets. Finally, we are using zebrafish to test candidate enhancer elements bound by SOX2 in dLGN chromatin, by assessing their ability to drive fluorescent reporter expression during early zebrafish development.

Overall, this work aims to define the SOX2–NR2F1 gene regulatory network underlying thalamic sensory nucleus identity and visual circuit formation, providing new insights into the molecular basis of neurodevelopmental visual disorders.