Guang Yang- Scientific Summary

Project Title: Dissecting and Targeting mRNA Regulation in Brain Development

Statement of Purpose: We aim to understand how mRNA translation is orchestrated to drive neural stem cell fate decisions during brain development and harness the underlying mechanisms to fight against neurodevelopmental disorders.

Project Summary:

The development of the mammalian brain requires balanced self-renewal and differentiation of neural stem/precursor cells (NPCs). Disrupting this balance causes brain malformations and neurodevelopmental disorders. While the expression of cell fate genes dictates the fate choice of NPCs, we find genes that promote differentiation are actively transcribed even before these cells decide to differentiate. The actual protein output of cell fate genes must therefore be carefully controlled at the translational level. However, what triggers the activation of cell fate mRNAs for timely, accurate fate decision-making is poorly understood.

In this project, we will address this question, inspired by recent insights we have gained from patients with a rare, previously uncharacterized neurodevelopmental disorder. These patients show malformations of the cerebral cortex and carry single missense mutations in the CELF2 gene that encodes an RNA-binding translational repressor shuttling between the nucleus and cytoplasm. Our initial studies in the mouse model show that: (i) CELF2 is localized in the cytoplasm of NPCs but translocates into the nucleus upon differentiation, (ii) Cytoplasmic CELF2 binds pro-differentiation mRNAs, and (iii) Pathogenic CELF2 variants fail to translocate into the nucleus. Thus, we hypothesize that cytoplasmic CELF2 represses cell fate mRNAs to maintain NPCs, and its nuclear translocation triggers the release of mRNAs for translation to induce differentiation, which, when disrupted causes cortical malformations. This also raises the possibility that targeting CELF2 transport might have therapeutic potential for neurodevelopmental disorders.

Aim 1: Determine how CELF2 translocation coordinates translation in NPCs for fate decision-making.
We will use conditional CELF2 knockout mice and a knock-in mouse line that harbours a patient-derived mutation to determine how loss- and gain-of-cytoplasmic CELF2 affects the translation of cell fate mRNAs, by using polysome profiling and RNA-sequencing. We will determine the impact of CELF2 perturbations on the balance of NPC self-renewal and differentiation in the developing cortex, by using immunohistochemistry and confocal microscopy.

Aim 2: Identify the signalling pathways that modulate CELF2 translocation to guide the development of potential therapeutic strategies.
Our initial study shows that the CELF2 mutant can be re-localized into the nucleus by an inhibitor of poly-ADP-ribose (PAR) polymerases that catalyze the addition of PAR to target proteins. We will determine how post-translational modification by PAR regulates CELF2 transport for NPC fate decisions, using genetics and cell biology approaches in both mouse and

human cell models. We will also use patient-derived NPCs to evaluate the effect of the inhibitor and other clinically used drugs on CELF2 localization and mRNA translation, by using confocal microscopy and translatomic analysis.

We have assembled a multidisciplinary team of both early career and established investigators with complementary expertise to conduct this project. This study will advance not only our understanding of RNA regulation in brain development, but will also reveal the mechanistic basis of a neurodevelopmental disorder and provide insight into potential treatment strategies.