Sarah Hughes-Scientific Summary

Project Title: Investigating how SMARCB1 post-transcriptional regulation regulates normal brain development is linked to development of autism

Statement of Purpose: We found that mRNAs encoded by the SMARCB1 gene family are regulated directly in neural stem cells. We will determine the mechanism of post-transcriptional SMARCB1 regulation including the proteins required.

Project Summary:

There is considerable evidence that autism spectrum disorder (ASD) originates during prenatal development as neural stem cells (NSCs) form the brain. Animal studies show that the normal role of identified ASD-risk genes is to regulate NSC proliferation / differentiation. Notably, chromatin remodelers constitute the majority of known ASD risk genes but their specific role in brain development is not well understood. SMARCB1 (SWI/SNF Related, Matrix Associated, Actin Dependent Regulator Of Chromatin, B1), is a subunit of the SWI/SNF chromatin remodeling complex. SMARCB1 mutations are common in patients with Kleefstra syndromes, neurodevelopmental disorders mirroring clinical aspects of ASD. We found that the SMARBC1 orthologue Snr1 (Snf5-related1) is required for differentiation of Drosophila NSCs called neuroblasts (NBs). Snr1 loss causes NBs to remain undifferentiated, a previously unknown role during brain development. We have also linked cell proliferation regulators like the tumour suppressor/actin binding protein Merlin to mRNA regulation via interaction with a non-canonical cap binding protein, eIF4E-3. RNA immunoprecipitation (RIP)-Seq of Merlin/eIF4E-3 complexes identified Snr1/SMARCB1 as the number one hit in both flies and mammalian neurons. The potential role for post-transcriptional regulation of Snr1 mRNA is strengthened by our findings that NB defects caused by loss of Snr1 cannot be rescued by Snr1 cDNAs missing the 5' or 3' UTR. cDNAs lacking the UTRs can rescue Snr1 activity in non NB cells. We also saw that Snr1 transcript levels in the brain remain unchanged in Merlin null mutant but Snr1 protein levels were reduced, suggesting regulation of Snr1 translation. Finally, we found that Snr1 mRNA is enriched at the apical NB cortex, but in Merlin mutants, localization is lost. We hypothesize that post-transcriptional regulation of localized Snr1 mRNA directs NSC proliferation and differentiation during early brain development.

Objective 1: Determine how Snr1 mRNA localization affects Snr1 protein activity. Localization of Snr1 mRNA is likely a mechanism to regulate Snr1 protein entry into the nucleus. We will first test the how directed protein transport affects NB differentiation using transgenes that express protein that is exclusively nuclear or cytoplasmically localized. The role of specific regions of the Snr1 mRNA (ie 3' UTR) in this process will be similarly tested by expressing Snr1 coding regions with or without UTRs. Finally, several other differentiation specifying proteins are cortical localized in NBs. We will test how mutations of these affect Snr1 localization using fluorescent in situ hybridization (FISH).

Objective 2: Identify mechanisms mediating Snr1 post-transcriptional regulation. RNA pulldown/mass-spectrometry using different regions of the Snr1 mRNA specifically identified actin binding, translation regulation and RNA binding proteins. For each candidate, the effect of

mutations on Snr1 localization (FISH), expression (western blotting) and NB differentiation and proliferation (counting cells expressing specific lineage markers) in whole brains.

Objective 3: Once confirmed in our simple fly model, candidates will be re-tested for a similar role in mouse brain development and behavior with our collaborators. Chromatin remodeling proteins are an active target of drug development. Characterization of this novel mRNA regulation of Snr1/SMARCB1 during brain development will provide additional therapeutic options for ASD patients.