Thesis Synopsis

Cerebellar granule neuron progenitors (CGNPs) give rise to a homogenous group of neurons called the cerebellar granule neurons in the developing cerebellum (a hindbrain structure classically associated with fine motor movements). These neurons constitute the single largest population of neurons in the brain. Generation of such large number of neurons is made possible by the intense proliferation of CGNPs in a transient layer called the external granule layer (EGL) in the dorsal cerebellum. A complete understanding of the factors which regulate proliferation of CGNPs in the developing cerebellum is lacking.

We found that the co-transcription factor, β-catenin, tends to be preferentially localized to the nucleus in proliferating CGNPs and gets asymmetrically distributed as the progenitors start exiting the cell cycle. This suggested that β-catenin could be a possible cell fate determinant in CGNPs. Upregulation of the protein via GSK3β inhibition and viral transduction showed that β-catenin indeed maintains CGNPs in a state of proliferation. We further show that the C-terminal transcription factor binding domain of the protein is essential for this function. Surprisingly, downregulation of the protein does not have any significant impact on CGNP proliferation.

β-catenin is normally regulated by the canonical Wnt pathway. We show that some of the most common canonical Wnts are expressed in the EGL however, none of them are able to alter β-catenin levels. Interestingly, β-catenin levels were found to be regulated by the mitogen Sonic hedgehog (Shh). Shh signaling led to an increase in the level of the active pool of β-catenin both in the cytoplasm and the nucleus of CGNPs. Shh induced increase in beta-catenin levels was independent of any canonical Wnt signaling. Next, we observed that Shh signaling regulates beta-catenin at the transcriptional level and it does so through the transcription factor N-myc. We found that N-myc binds to the β-catenin promoter and modulation in N-myc levels leads to concomitant changes in β-catenin expression. Thus, we propose that Shh signaling activates β-catenin mediated proliferation in CGNPs. To the best of our knowledge, this is a novel signal transduction pathway in CGNPs.

We also investigated the role of centrosome inheritance in CGNP proliferation. Centrosomes have an inherent asymmetry wherein one of the centrioles is older than the other. Studies have shown a correlation between the age of the centriole inherited by a daughter progenitor and the cell fate is subsequently adopts. Though we found inherent asymmetries in the centrosomes in CGNPs, we failed to observe any pattern in inheritance of centrosomes related to cell fate determination. This suggests that the role of centrosomes in progenitors may not be conserved across cell types.