Neurogenin2 Regulation of Cell Cycle Progression during Mouse Retina Neuron Formation

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Introduction:

Neurogenin2 (Neurog2) is a bHLH factor that regulates expansion of neurogenesis and cell differentiation and survival. Knockout mutants express a developmental delay in the accumulation of retinal neurons, which is subsequently rescued. The molecular mechanism for this delay is unclear.

Aims/Hypotheses:

We hypothesized that an increase in cell cycle length in progenitor cells causes a delay in progression to cell cycle exit and differentiation in mutants compared to controls. We assessed this by comparing cell cycle lengths by mitotic window labeling on Neurog2 mutants and heterozygotes.

Methods:

Neurog2 heterozygote (Het) and knockout (KO) littermates were compared. A single injection of BrdU was administered at E11.5 and embryos allowed to develop for another 48 hours. E13.5 embryo heads were collected, fixed in PFA/PBS, embedded in OCT, and sectioned at a thickness of 10 μm. Immunohistochemistry was performed using anti-GFP, anti-BrdU, and anti-Ki67 antibody labeling. Microscopy was performed with a Zeiss fluorescent microscope, Zeiss digital camera, and Apotome deconvolution device and quantified using Adobe Photoshop CS4. Sections were analyzed for the number of total mitotic retinal progenitor cells (RPCs) at time of BrdU injection (BrdU+GFP+ cells), the number of RPCs that stayed mitotic throughout the 48 hour window (Ki67+BrdU+GFP+) and the number of RPCs that exited the cell cycle during the 48 hour window (Ki67-BrdU+GFP+).

Results:

Eight embryos (4 Het, 4 KO), from 4 different litters were evaluated. The ratio of RPCs that stayed mitotic after BrdU injection of total mitotic RPCs at time of BrdU injection (Ki67+BrdU+GFP+ cells/total BrdU+GFP+ cells) was found to be significantly increased in KO subjects compared to control. There was also a significant decrease of the ratio of RPCs that exited the cell cycle after BrdU injection of total mitotic RPCs at time of BrdU injection (Ki67-BrdU+GFP+ cells/total BrdU+GFP+ cells) in KO mice as compared to control.

Summary/Conclusions:

There is a significantly larger population of RPCs stuck in the mitotic cycle from E11.5 to E13.5 in the KO mice as compared to controls, suggesting that the developmental delay observed in Neurog2 mutants is due to a prolonged cell cycle. Further studies are necessary to pinpoint when in the cell cycle these RPCs are being delayed and to determine which cell cycle machinery factors Neurog2 normally regulates.

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