Genetic Mechanisms of Retinoblastoma
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Introduction
Retinoblastoma is a type of cancer that occurs early in child development. This disease is rare, with only 1 out of 20,000 children affected (Genes and Disease). Why study such a rare disease? Metastatic retinoblastoma is among the most deadly of childhood cancers with a less than 10% probability of survival. Secondly, studies in retinoblastoma have paved the way for understanding many of the molecular interactions that drive tumorigenesis in other tissues. Retinoblastoma is associated with mutations of the Rb gene locus. Rb is a tumor suppressor gene, which functions to regulate the cell cycle, and mutations of the Rb locus can lead to cell proliferation. The Rb protein regulates the cell cycle by binding to the E2F transcription factor. The E2F transcription factor is involved with cell cycle progression (proliferation) but also has a key role in programmed cell death, also known as apoptosis (Hallstrom). Understanding how mutations of the Rb locus is involved with cell proliferation will likely be useful for diagnosis and pharmacological treatment of many cancer types.

Mouse Genotypes:
1. Chx10 Cre – control.
   • Cre is expressed in retinal progenitor cells.
2. Chx10 cre; Rb flox/flox; p107-/-
   Experimental group.
   • Rb and p107 knockout leads to high level of E2F activation in the retina

Experimental Design
1. Determine genetic background of mice via Polymerase Chain Reaction (PCR).
2. Isolate RNA from retinal cells of newborn mice.
3. Perform quantitative PCR on isolated retinal RNA. E2F levels were found to be unchanged.
4. Isolate retinal RNA from embryonic day 14 pups. These mice have been shown to have a higher percent of GFP/Cre+ cells in the retina.
5. Perform qPCR on embryonic day 14 retinal RNA. E2F levels were also found to be unchanged.
6. Isolate retina of newborn mice and separate GFP/Cre+ cells from GFPCre- cells via fluorescence activated cell sorting. Isolate RNA from GFP+ cells.
7. Perform qPCR on GFP+ cell RNA. Our data showed E2F expression levels to be around a seven fold increase as compared to the control.

Future Directions
• Determine if E2F activation induces expression of E2F target genes in vivo.
• Determine if changes are affected by the mutational status of the Pten locus, which was shown by our laboratory to suppress E2F’s apoptotic function in vitro.
• Explore the possibility of treating Rb/PTEN mutant tumors with targeted inhibition of the PI3k/AKT signaling pathway which will restore E2F’s apoptotic function and thereby destroy the cancerous cells.

Bibliography