Retinal Cell Biology

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Goals and projects

Research in the laboratory of Brian D. Perkins, Ph.D., uses zebrafish and induced pluripotent stem cell (iPSC) models to study photoreceptor degeneration and regeneration.

Inherited retinal diseases lead to irreversible loss of rod and cone photoreceptors and frequently result in blindness. Leber's Congenital Amaurosis (LCA) is the most common blinding disorder in children. Unfortunately, the body has no way to replace those cells once they are gone. Mutations in the gene for *Centrosomal Protein of 290 kDa* (*CEP290*) are one of the leading causes of LCA, as well as other ciliopathies such as Joubert Syndrome (JBTS) and Bardet-Biedl Syndrome (BBS). Importantly, photoreceptor degeneration is one of the most common manifestations in ciliopathies. The long-term goals of this laboratory are to understand the mechanisms regulating the formation of photoreceptor outer segments and to understand how to regenerate photoreceptors in genetic models of retinal degeneration. Our laboratory utilizes a complementary approach of zebrafish mutants in *cep290* and several other cilia genes, as well as 3D retinal cups generated from patient-derived human iPSCs. Zebrafish *cep290* mutants undergo retinal degeneration and we are exploring the mechanisms that lead to photoreceptor loss and attempting to identify signals that can trigger regeneration in these mutant zebrafish. We are also using iPSCs created from patients with *CEP290* mutations to make retinal organoids (also known as "retinas-in-a-dish") in order to establish the mechanisms that determine disease severity. Our goal is to combine knowledge from stem cell derived retina and zebrafish to develop better therapies for LCA patients in order to restore vision.

Research & Innovations

Studies

1.) Zebrafish models of childhood blindness

1. Genetic and functional studies of during photoreceptor cilia formation

Personnel: Brian Perkins, Ping Song, Joe Fogerty, Rachel Stupay

Mutations in the gene for *Centrosomal Protein of 290 kDa* (*CEP290*) result in several different diseases that exhibit a range of clinical symptoms, which typically include retinal degeneration. The Cep290 protein localizes to the ciliary transition zone (TZ), the region of the cilium located between the basal body and microtubule axoneme. The TZ serves as a diffusion barrier or gatekeeper for passage of cilia proteins and corresponds to the connecting cilium of vertebrate photoreceptors. Cep290 is believed to be required for proper assembly and function of the TZ but how loss of Cep290 leads to retinal degeneration is unclear. Mutations in *CEP290* are a cause of Leber Congenital Amaurosis, an early form of childhood blindness, but can also cause more pleiotropic diseases such as Joubert Syndrome and Bardet-Biedl Syndrome. *Although photoreceptor survival requires Cep290, the precise function of Cep290 has not been clearly identified and the mechanisms leading to photoreceptor death remain unknown. Furthermore, no there is no consensus view that explains why mutations in a single gene, such as CEP290, result in such variable disease phenotypes*. It has been hypothesized that phenotypic severity reflects the genetic interactions between mutations in specific genes (e.g. *CEP290*) with heterozygous mutations in other cilia genes present within the genetic background. These second-site "genetic modifiers" can enhance disease penetrance or severity. Alternatively, it was recently proposed that disease severity correlates with *total* protein levels of Cep290. In a process known as nonsense-mediated alternative splicing, exons harboring nonsense mutations are selectively skipped from the final transcript. When exon skipping does not alter the overall reading frame of the final transcript, moderate levels of near-full-length protein can be produced, thereby resulting in less severe phenotypes.

2.) Induced pluripotent stem cell (iPSC) models of retinal degeneration

2. Basal exon skipping of *CEP290* in human derived iPSCs and 3D retinal organoids

Personnel: Brian Perkins, Joe Fogerty

The highly variable nature of *CEP290*-associated disease phenotypes cannot be explained by traditional genotype-phenotype correlations. One possibility is that exons harboring nonsense mutations and that also begin and end in the same reading frame can be preferentially skipped. In such a case the resulting mRNA transcript eludes nonsense-mediated decay and can produce a near-full-length protein. Disease severity therefore correlates with the total amount of full-length and near-full-length protein produced. We will take blood from patients with *CEP290* mutations and generate human iPSCs and subsequently differentiated into 3D retinal cups, or retinal organoids. These retinal organoids will be used determine if basal exon skipping occurs in from humans carrying *CEP290* mutations and whether total protein levels correlate with disease severity. In addition, how disease-causing mutations lead to alterations in cilia architecture and protein trafficking will be investigated. These experiments will establish the molecular and genetic mechanisms that determine the severity of disease progression.

3.) Identifying the mechanisms regulating retinal regeneration

3. Retinal regeneration in zebrafish *cep290* mutants

Personnel: Brian Perkins, Ping Song, Joe Fogerty, Rachel Stupay, Lauren Cianciolo

In response to retinal injury, zebrafish exhibit a robust capability of regenerating lost neurons, including photoreceptors. Retinal damage causes release of growth factors and inflammatory cytokines that trigger Muller glia to divide and generate multipotent retinal progenitor cells that regenerate lost neurons. Central to this process is a reprogramming event that involves activation of Stat3. While robust regeneration occurs following widespread acute injuries, evidence from the literature and preliminary data here indicate that regeneration can be disrupted in zebrafish with inherited forms of retinal degeneration. These observations suggest that Muller glia respond differently to widespread acute vs. progressive inherited forms of retinal injury and that regeneration is only triggered when the degree of retinal injury crosses a “damage threshold” within a given temporal window. Systematic testing of this hypothesis has not been previously attempted and the differential response of Muller glia to progressive degeneration remains a significant gap in knowledge. We therefore propose to evaluate signaling pathways that are relevant to retinal regeneration in *cep290* mutants, identify deficiencies, and then stimulate these pathways to evoke a regenerative response from Muller glia.

Lab staff members:

- Brian D. Perkins, PhD, *Director*
- Ping Song, PhD, *Project Staff*
- Joe Fogerty, PhD, *Project Staff*
- Rachel Stupay, PhD, *Postdoctoral fellow*
- Lauren Cianciolo, *Post baccalaureate Scientist*