Optimizing the Intermedilysin (ILY)- hCD59 Receptor System of Rapid Cell Ablation in Zebrafish

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Targeted cell ablation is a powerful and important tool for studying cellular processes. Yet current methods are slow, requiring hours to days, making them unsuitable for studying rapid cellular events lasting seconds to minutes. The Chandrasekhar lab is testing a novel rapid cell ablation technology, hCD59-ILY, for feasibility in zebrafish. To do so, the lab generated a transgenic zebrafish line *Tg(zCREST:ZsGreen-P2A-hCD59)* that expresses the human CD59 receptor and zsGreen fluorescent protein in branchiomotor neurons. While preliminary cell culture studies demonstrated that these neurons undergo rapid cell lysis following treatment with ILY protein, the conditions in vivo remain to be optimized. My project will test several combinations of hCD59 receptor and ILY protein concentrations to determine the optimal expression levels for efficient cell ablation in zebrafish embryos.

Tg(zCREST:ZsGreen-P2A-hCD59) zebrafish will be set up for breeding and embryos will be collected for treatment, and a range of concentrations of mRNA and ILY will be tested. These embryos will be injected with hCD59 mRNA into the yolk syncytial layer (YSL) of the embryo, where necrotic regions first form, and then incubated in differing levels of ILY protein. The ILY-treated embryos will be examined to determine which combination produces the highest percentage of embryos with necrotic region formation.

This summer, strides have been made in this project by optimizing injection technique, timing, and preparing for testing. This summer, embryos have been injected with GFP mRNA in differing concentrations to ensure proper expression in the YSL and differing levels of fluorescence. During the academic year, we will begin injections of hCD59 and ILY treatment experiments.

I expect an optimal combination of hCD59 receptor and ILY protein to be found that causes maximal tissue lysis, making such a combination both efficient and effective. These findings can then be used for neuroscience and developmental biology research.