SURF Research Proposal Form

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Project Title: Identifying and Characterizing a Genetic Modifier for Cataracts
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1. Research Statement

Cataract is the clouding of the lens of the eye. According to the World Health Organization, cataract is the leading cause of blindness worldwide. Currently, the only effective treatment to remove cataracts is surgery, which costs $6.8 billion annually in the United States. Understanding the underlying mechanisms that cause cataracts may lead to new therapeutic strategies to prevent or delay the progression of cataracts, thereby alleviating the need for surgical intervention and possibly saving billions of dollars. Gap junctions are membrane channels that connect adjacent cells, allowing the transfer of metabolites, ions, and other small molecules for maintaining normal transparency of the lens. Previous research from the Gong Lab has shown that the deletion of alpha3 connexin, a primary component of lens gap junctions, results in severe cataracts in the 129SvJae (129) mouse strain but displays mild cataracts in the C57BL/6J (B6) mouse strain. My proposed research project seeks to identify and characterize a novel genetic modifier on mouse chromosome 2, which is responsible for suppressing cataract severity. Based on current knowledge of these identified cataractous suppressors, I hypothesize that this novel genetic modifier functions as a cytoskeleton-associated protein to influence cataract formation in the lens.

2. Background to the Topic and Rationale for Your Research

The lens is a multicellular transparent organ mainly consisting of long fiber cells covered by a single anterior surface layer of epithelial cells. Cells in the lens communicate through gap junction channels formed by membrane proteins called connexins. The lens expresses three main isoforms of connexin: alpha1 (connexin43 or Gja1), alpha3 (connexin46 or Gja3), and alpha8 (connexin50 or Gja8). Mutations in the alpha3 connexin gene are a common cause of cataracts in humans (Addison et al., 2006; Hansen et al., 2006). Therefore, it is important to understand the molecular mechanism and role of alpha3 connexin in gap junction communication and cataract formation. Genetic studies have demonstrated that lenses of alpha3 knockout mice develop nuclear cataracts, affecting the transparency of the center of the lens. This cloudiness is due to the aggregation of proteins in the lens, which is caused by the cleavage of gamma-crystallin detected in the lenses of alpha3 knockout mice (Gong et al., 1997). However, various mouse strains present differently to the same deletion. Alpha3 knockout in the 129 mouse strain resulted in severe cataracts associated with gamma crystallin cleavage, whereas alpha3 knockout in the B6 mouse strain led to far milder cataracts with no detectable gamma crystallin cleavage, suggesting that there are genetic modifiers that influence the severity of cataracts in the absence of alpha3 connexin (Gong et al., 1999). The Gong Lab has identified two genetic modifiers for

nuclear cataract formation: periaxin and CP49. Disruption of periaxin, a cytoskeletal scaffolding protein, disturbs the packing of lens fiber cells (Maddala et al., 2011). The knockout of CP49, an intermediate filament protein, destabilizes the cytoskeleton of lens fiber cells (Sandilands et.al, 2003). The combined effects of gap junctions, periaxin, and CP49 regulate the function of lens fiber cells to control lens transparency. However, there is still not a complete understanding of how the knockout of alpha3 can lead to various cataract severities in different mouse strains. We have mapped a novel genetic modifier to mouse chromosome 2. Because the previous two genetic modifiers detected have been related to the cytoskeleton of fiber cells, I hypothesize that this third genetic modifier may also function as a cytoskeleton-associated protein. The goal of my project is to identify and characterize this unknown modifier for understanding the pathological mechanism behind the varying opacifications of the different mice strains in the absence of alpha3 connexin.

3. Research Plan

This research project seeks to determine a novel gene that acts as a genetic modifier for cataract severity in the absence of alpha3 connexin. I will continue to use mice as a model organism for studying cataract formation because mouse lens and human lens share many features in anatomy, biochemistry, and development. As I will need to obtain lenses from B6 and 129 mouse strains, phase 1 of my project will involve backcrossing our current mice to further narrow down the chromosomal region where this unknown genetic modifier is localized. I will take tissue samples from these mice and perform PCR and gel electrophoresis to determine their genotypes. The duration of phase 1 will last the entire summer as I will continually need to analyze new lenses from the mice of my desired genotypes. The results from this phase will determine if I need to continue breeding mice or if I can move forward with the characterization of the lens pathology of alpha3 knockout mice in my next phase.

In phase 2, occurring from weeks 2 through 4, I will identify the gene that functions as the genetic modifier suppressing nuclear cataracts in the B6 mouse strain. This gene will have to display genetic variances between the 129 and B6 mouse strains. In addition, to support my hypothesis, this gene should also encode for a protein relevant to lens membranes and cytoskeleton complexes. The Gong Lab has already predicted candidate genes on an interval on mouse chromosome 2 based on the genetic variances in the mouse genome database. By searching in the most updated SNP mouse genome database, I will continue to search for additional candidates. I will perform RT-PCR to verify the expression profiles of the candidate genes in the lens to find genes that support my hypothesis.

Once I select a suitable candidate gene, I will proceed to phase 3 in which I will use a variety of imaging techniques to visualize the distribution and locations of this candidate protein in the lens fiber cell during development. During weeks 5 and 6, I will first take images of whole lenses with a dissecting microscope to identify any observed light scattering. These images will help me evaluate the severity of the cataracts and will provide me a general location of where the light

scattering occurs within the lens. Then in weeks 7 and 8, I will perform a histological analysis of the lens to focus closer on the nuclear and cortical regions to determine a more defined location of where proteins are aggregating. In addition, in weeks 9 and 10, I will cut sections of the lens and perform immunofluorescence staining to see if there are changes to the morphology of the lens. Because I hypothesized that the candidate protein will have functional relevance with membrane and cytoskeleton complexes, I will search for changes in the internal organization of lens cells. By analyzing images of lenses from mice of different ages, I will be able to examine how this gene impacts lens development and cataract formation.

In phase 4, occurring during weeks 11 and 12, I will test to see if or how the function of the candidate protein is associated with crystallin degradation in cataract formation. If this genetic modifier functions to suppress cataracts in the B6 mouse strain, I expect there to be a decrease in crystallin degradation and will use Western blot analysis to confirm this hypothesis. In phases 3 and 4, I hope to determine how differences in the 129 and B6 mouse strains alter the properties of the candidate protein in the context of gap junction communication and cataract severity.

I will fortunately have the guidance of Professor Xiaohua Gong and Dr. Chun-hong Xia throughout the entire summer to carry out this project. At the end of each week, I will have a formal meeting with Dr. Xia to share and discuss my results. In addition, I will meet with Dr. Gong after the completion of each phase of the project to update him on my progress and receive feedback. Through my planned timelines, methodologies, and support in the lab, I believe I can realistically and successfully carry out my proposed research project.

4. Qualifications and Project Affiliations

As an undergraduate student majoring in Molecular and Cell Biology, I have developed a deep interest in studying the molecular complexity of the human body. Having received strong grades in all of my science and lab courses, I believe I have a solid understanding of cellular processes and am prepared to think like a researcher in tackling difficult questions in science. Seeing my grandparents struggle with their declining vision due to cataracts, I joined the Gong Lab in January of 2020 and developed a deeper understanding of the molecular mechanism behind cataracts. Under the supportive mentorships of Professor Xiaohua Gong and Dr. Chun-hong Xia, I have had engaging discussions about the anatomy, biochemistry, and physiology of the lens. I have also acquired extensive experience conducting literature searches and reading scientific papers to expand my knowledge. I have fine-tuned my laboratory techniques in PCR, gel electrophoresis, Western blot, lens dissection, and immunostaining. In addition, I have already received the proper training and authorization from the Office of Laboratory Animal Care (OLAC) to work with laboratory mice. Research has shown me how science requires curiosity, perseverance, and tenacity, which are all qualities I use in any challenge presented to me. In addition, as a leader of multiple campus and community groups, I have developed communication, organization, and problem-solving skills. From my lab experiences, training, coursework, activities, and connections, I believe I have the resources and necessary skills to

thrive as an independent researcher and successfully carry out my own summer research project.

5. Citations and Core Texts

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