Research Statement

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Dysfunction and subsequently death of photoreceptors is a major cause of incurable vision impairment in retinal degenerative diseases. Currently, only a few treatment options are available. This is, in part, due to the lack of appropriate model systems to investigate mechanisms of human disease pathogenesis and rapidly evaluate new treatments. Gene or cell replacement therapies, along with neuroprotection, have shown promising results in ameliorating photoreceptor dysfunction in some instances. However, these therapies still face safety concerns and technical/fiscal challenges that must be overcome before reaching patients worldwide. My research interests have evolved during the years of my graduate and post-doctoral training. As an independent investigator, I will focus on developing functional 3-D retinal organoids from pluripotent stem cells and investigate human retinal development and disease, with a goal to evaluate novel therapies.

Previous Research (Graduate Studies; Mentor: Dr. Nelson Leung-Sang Tang)

My graduate studies focused on delineating the association between circulating insulin-like growth factor 1 (IGF1) levels and genetic polymorphisms in its evolutionarily-conserved promoter region. The research experience provided me the requisite training of molecular genetics and in the broader scientific discipline how to think and design the experiments and analyze the data. Fascinated by rapid developments in stem cell technologies and enormous potential of 3-D organoid culture system for therapeutic purposes, I decided to focus on stem cell-based research for my postdoctoral training.

Current Research (Post-doctoral Studies; Mentor: Dr. Anand Swaroop)

Improvement of mouse and human organoid cultures: At NEI/NIH, I have developed novel approaches for both mouse and human retinal organoid differentiation to more accurately recapitulate the *in vivo* retina. The low efficiency of mouse retinal organoid differentiation was a major hindrance, which I was able to overcome using hypoxia conditioning to mimic the microenvironment during mouse retinal development. This higher efficiency differentiation enabled me to supervise a group of bioengineering post-baccalaureate students for establishing the first reported culture of retinal organoids in bioreactors. These organoids demonstrated higher viability of retinal ganglion cells and cone photoreceptors, the cell types involved in common retinal diseases. Additionally, I have optimized protocols for human retinal organoid differentiation by replacing widely-used all-*trans* retinoic acid (the current standard) with 9-*cis* retinal in the culture system. The 9-*cis* retinal-supplemented organoids exhibit expedited and more mature differentiation of rod photoreceptors.

I have used these advancements to model Leber congenital amaurosis (LCA) caused by *CEP290* mutations in patient stem cell-derived retinal organoids, which display many disease-associated phenotypes including compromised cilia development and opsin mis-localization in photoreceptors (Fig 1). My current and future studies (see below) explore both basic and translational aspects of organogenesis, disease mechanisms, and novel therapies for retinal degeneration, by recapitulating physiological events in a dish.

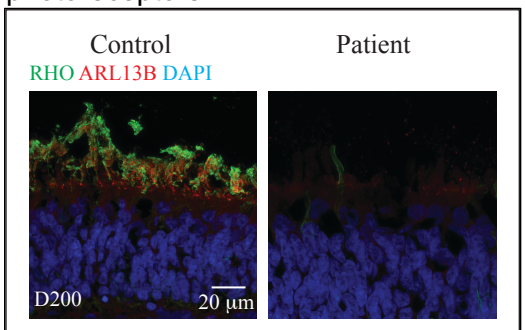

Fig 1. Immunohistochemistry analysis of D200 control and patient induced pluripotent stem cell-derived retinal organoids. Rod photoreceptors and cilia are indicated by Rhodopsin (RHO, green) and ADP-ribosylation factor-like GTPase 13B (ARL13B, red), respectively.

Development of novel therapies for retinal degenerative diseases: As photoreceptor differentiation in human stem cell-derived retinal organoids requires as many as 200 days, such systems pose significant technical challenges for the design of high-throughput assays for small molecule screening to rescue disease phenotypes. I have therefore employed mouse retinal organoids, which require only 30-35 days in culture, to fulfill this objective. In collaboration with scientists at the National Center for Advancing Translational Sciences, we have designed a multiplex screening platform using mouse organoids from a *Cep290*-mouse model to select drug candidates that maintain photoreceptor survival. Positive hits are now being evaluated using human *CEP290*-LCA organoids as proof-of-concept evidence for potential therapeutic application.

I am also collaborating with Dr. Zhijian Wu's group at the National Eye Institute to design gene therapies using adeno-associated viruses (AAV) for *CEP290*-LCA. Small packaging capacity of AAV makes

vector design difficult for full-length *CEP290*. Therefore, I am evaluating AAV vectors carrying overlapping CEP290 domains to ameliorate the phenotypes in patient-derived retinal organoids. My studies thus utilize *in vitro* stem cell-based retinal organoid system as a model for evaluation of novel therapies.

Future Research Goals

I envision my future research to focus on generation of a more comprehensive and functional organoid culture system and apply this platform to investigate retinal development and disease for translational goals.

Pathogenesis of retinal degeneration in *CEP290*-LCA patients: Human retinal organoids differentiated from *CEP290*-LCA patient iPSCs exhibit ciliary defects and opsin mis-localization in photoreceptors. However, it is unclear how these defects ultimately lead to photoreceptor degeneration. Our initial studies have revealed aberrant ciliary trafficking and endoplasmic reticulum (ER) to Golgi transport, along with mitochondrial dysfunction in patient photoreceptors. I hypothesize that photoreceptor cilia defects disrupt protein trafficking and trigger unfolded protein response (UPR) in ER, which subsequently leads to mitochondrial dysfunction and cell death. I will first compare the UPR between control and patient retinal organoids and investigate the signaling pathways by molecular and biochemical assays. I will also investigate the interaction between ER and mitochondria, with a focus on calcium signaling and molecular chaperones. Given that comparative transcriptome analysis has uncovered abnormal expression of genes which may trigger immune response, I will establish co-culture system of retinal organoids and the primary immune cells in the retina, microglia, to evaluate their interaction during retinal degeneration. The final goal of the study would be to identify the triggers of photoreceptor degeneration and perform high-throughput screening to identify potential targeted genes/signaling pathways to maintain photoreceptor survival.

Modeling diabetic retinopathy in human organoid cultures: Diabetic retinopathy is a major cause of blindness, but the etiology of the disease is still under debate. I plan to investigate how human retina is affected by the disease-causing environmental and genetic perturbations by modeling the disease in retinal organoids. I will initially focus on examining the impact of glucose concentrations on organoid cultures and investigate their interactions with other cell types/ tissues by co-culture with glial cells and/or organoids with endothelial cells. I would also collaborate with clinician scientists to obtain iPSCs from diabetes patients with or without retinopathy and compare their development in organoid culture. I will perform high-throughput RNAi screening to identify the genes/signaling pathways responsible for the maintenance of normal functions of photoreceptors under diabetic conditions. These studies would complement ongoing biochemical and genetic investigations in other laboratories, provide useful information for understanding pathophysiology of diabetic retinopathy, and assist in evaluation of therapies.

Generation of functional synapses in human retinal organoids: Improvement of patient vision by cell replacement therapies requires the formation of functional connections between transplanted cells and host interneurons. However, the underlying mechanisms for synaptic wiring remain largely unclear. Notably, retinal degeneration leads to reorganization of neural circuits and alterations in the inner retina. 3-D retinal organoids contain retinal cell types organized into a laminated architecture mimicking their *in vivo* counterparts and offer a unique opportunity to study synaptogenesis in retinal development and disease. However, despite the presence of multiple retinal cell types, synapses in plexiform layers of retinal organoids do not display mature morphology. At this stage, molecular aspects of neural circuit formation between the outer and inner retina neurons are poorly delineated. We hypothesize that functional synapses could be established by providing *in vivo*-like microenvironment coaxed by appropriate intrinsic and extrinsic factors. I aim to: (1) establish imaging modalities to evaluate synaptic activity in organoid culture, by expressing channelrhodopsin in photoreceptors and cell-type specific fluorescent markers and GCamP in bipolar cells; (2) generate functional synapses in human retinal organoids. I will evaluate the impact of intrinsic genetic or epigenetic factors (by high-throughput RNAi screening in collaboration with NCATS), extrinsic factors (by co-culture with microglia, which have been shown to be implicated in synaptogenesis in retina), and biophysical approaches (by providing electric oscillation impulse to simulate spontaneous rhythm generated by RGC, which cannot be maintained in organoid culture) to synaptic activities; and (3) model neural circuit in patient iPSC-derived retinal organoids and investigate the underlying pathology. My studies should generate a new model for investigating synaptogenesis, provide insights into regulation of synaptic development, and help in evaluating therapies for rescuing functional circuits in retinal diseases.