ARVO EDUCATION COURSE

Saturday, May 4, 2024
8am-4:30pm
Seattle Convention Center
Seattle, WA

Development of pluripotent stem cell-based platforms for disease modeling and therapies

Course organizers

Holly Chen, PhD, University of Alabama at Birmingham

Course description

Pluripotent stem cell-derived retinal tissues exhibit morphological resemblance and significant functional parallels to in vivo retinal structures, presenting a promising in vitro platform. Nevertheless, a notable disparity persists between the current maturity of this platform and its potential therapeutic applications. This course will comprehensively introduce various types of pluripotent stem cell-derived tissues and update the audience with the recent advances. It will also provide examples of how to apply pluripotent stem cell-based platforms to evaluate therapies. Finally, a panel discussion will provide an opportunity for networking and in-depth discussion between speakers and audience to address specific projects.

Learning objectives

Attendees will leave this session with the ability to:

- Describe various approaches to generate pluripotent stem cell-derived retinal tissues.
- Outline current advances and future directions in stem cell technologies.
- Describe the latest updates and developments in ARVO, AAO, and NEI initiatives for AI and big data in ophthalmology and visual sciences.
- Recognize the pros and cons of pluripotent stem cell-based approaches.
- Design pluripotent stem cell-based platform for developing therapies.

Presentations

Presenters and presentations may change.

Time	Topic	Speaker
8 AM	Differentiation of pluripotent stem cells into photoreceptors	Holly Chen, PhD, University of Alabama at Birmingham
	The limited number of cells in the retina and challenges in the maintenance of primary retinal cultures have hindered the progress of therapeutic development. Pluripotent stem cells have unlimited proliferation capacity and can be readily differentiated into numerous retinal cell types including photoreceptors, whose dysfunction and/or degeneration is a major course of retinal degenerative diseases. This lecture will describe the current approaches to generate	

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	photoreceptors from pluripotent stem cells and the cutting-edge technologies to improve the cultures. We will also introduce various approaches to characterize the cultures to determine their structure, molecular composition, maturity, and functionality. Lastly, we will discuss the strengths and weaknesses of this technique. After the lecture, attendees are expected to be familiar with various approaches to generate and characterize photoreceptors from pluripotent stem cells and understand the current progress of this technique.	
9 AM	Generation of retinal pigment epithelia (RPE) from PSC	Kapil Bharti, PhD, National Eye Institute
	Presenter will show protocol for differentiation of iPSC into RPE cells and also show methods to validate functionality of differentiated RPE cells.	
10 AM	Break	
10:15 AM	How to build an RGC: Strategies to direct the differentiation of human pluripotent stem cells into retinal ganglion cells	Jason S. Meyer, PhD, Indiana University
	Retinal ganglion cells (RGCs) serve as the sole connection between the eye and the brain and are the primary cell type affected in optic neuropathies including glaucoma. Numerous protocols have been established to derive RGCs from hPSCs, with many studies using these cells as a model for human RGC development and cell type specification and also as an *in vitro* disease model for optic neuropathies. Methods to derive RGCs have been varied, including both 3D and 2D cultures as well as both directed differentiation as well as transcription-factor driven approaches. Regardless of the differentiation method, however, certain key criteria must be established to effectively leverage stem cell derived RGCs as effective *in vitro* models. In this presentation, the differentiation of hPSC-derived RGCs will be discussed, as well as the many unique characteristics associated with these cells in vitro including their genetic identifiers, electrophysiological activity, morphological features, as well as transcriptional profiles. Also described will be the current progress in the use of patient-specific hPSCs to study optic neuropathies affecting RGCs, with emphasis on the use of these RGCs for studying disease mechanisms and pathogenesis, drug screening, and cell replacement therapies.	
11:15 AM	Using AMD patient iPSC-derived choroidal endothelial cells to evaluate disease pathology and develop an autologous cell replacement strategy.	Budd A. Tucker, PhD, University of Iowa
	Age-Related Macular Degeneration (AMD) is a common, blinding disease with limited treatment options. The high prevalence of AMD coupled with its severe toll on vision, make understanding	

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	its disease mechanisms and developing potential therapies part of our core mission. Although anti-VEGF therapies are effective in many cases of advanced neovascular AMD, the only intervention currently available for atrophic AMD – the most common form of the disease – is dietary vitamins. Death of choroidal endothelial cells is one of the earliest detectable pathologic features of AMD, often occurring prior to both RPE and photoreceptor cell loss. Although RPE cell dysfunction may precede and contribute to choriocapillaris dropout, early loss of choroidal endothelial cells is a major contributor to disease pathogenesis. Understanding what causes choriocapillaris dropout early in disease progression may enable development of novel therapeutics designed to prevent choroidal endothelial death and subsequent loss of RPE, and photoreceptors. Furthermore, development of choroidal endothelial cell replacement strategies, designed to repopulate the choriocapillaris in early AMD or as a part of a multilayer graft for advanced disease, is greatly needed. In this seminar I will present data to demonstrate development of induced pluripotent stem cell differentiation and enrichment protocols designed to enable production of patient specific choroidal endothelial cells. In turn, I will demonstrate how we are using iPSC-derived choroidal endothelial cells for modeling of disease pathology and autologous cell replacement.	
12:15 PM	Lunch	
1:15 PM	Panel: Open Q&A with presenters	
1:45 PM	Making the best cell: using stem-cell-derived retinal organoids to grow neurons for cell replacement therapies.	Petr Y. Baranov, MD, PhD, Harvard Medical School
	In this workshop, I will discuss recent advancements in the field of retinal organoid differentiation, including the application of automation and artificial intelligence for scaling up cell cultures. We will also review key transplantation studies that focus on the replacement of retinal ganglion cells.	
2:25 PM	Gene therapies	Jan Wijnholds, PhD, Leiden University Medical Center
	Gene therapies for patients, harboring autosomal recessive or dominant or X-linked inherited retinal disease mutations, which will become or already are blind needs optimization of the gene therapy strategies. Therapies developed on animal eye disease models or human cell lines might not work as efficiently in patients, therefore more suitable cultured human eye disease models are preferred in preclinical gene therapy testing. Discussed will be the different types of, and tools for, eye gene therapies. Gene therapies include gene augmentation that introduce a healthy gene, gene editing that corrects faulty genes or deletes unwanted DNA sequences, gene addition to introduce optogenetic sensors or another useful gene, gene silencing to inactivate dominant	

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	negative active genes, RNA therapy to introduce RNA or DNA antisense oligonucleotides or RNA aptamers or microRNA or small interfering RNA or messenger RNA or other RNA. In several of these procedures "vectors" are used to deliver the candidate medicine to the target cells. The vector can be viral or non-viral with the viral vectors lacking all of the viral expression sequences, or non-viral such as nanoparticles or virus-like particles. The viral vectors mostly used in gene therapy are derived from adeno-associated virus (AAV), adenovirus or lentivirus. AAV vectors with serotypes AAV2, AAV5, AAV8 or AAV2.7m8 and other newly engineered capsids are of major interest for use in therapy on the retina of patients because the AAVs have been shown to be efficacious and safe to target cell types in clinical studies and to achieve multiple years of function. The use of suitable promoters, with either cell-specific or ubiquitous activity, can enhance the expression in target cells. And finally, optimization of gene therapies on human pluripotent stem cell-derived retinal organoids and donor retinal explants will be presented.	
3:05 PM	Break	
3:20 PM	Application of pluripotent stem cell-derived cultures for drug discovery	Holly Chen, PhD, University of Alabama at Birmingham
	According to FDA Modernization Act 2.0 2023, which is recently signed by President Biden, animal tests are no longer needed before human drug trials. The utilization of specific alternatives including cell-based assays is now permitted by this comprehensive legislation. In this lecture, we will first describe the drug discovery pipeline for therapeutics and then demonstrate various approaches to establish drug screening platforms using pluripotent stem cell-derived cell types or tissues. Lastly, we will discuss the selection criteria of positive hits and the design of reliable secondary assays. After the lecture, attendees are expected to be able to design cell-based assays to identify effective therapeutics for retinal degenerative diseases.	
4:00 PM	Panel: Open Q&A with presenters	
4:30 PM	Adjourn	