Genome surgery and base editing: From biology to therapy

Organizer
Stephen H. Tsang, MD, PhD, Columbia University

Description
In the current era of personalized medicine, a large number of genetic variants in patients with various diseases have been identified using next-generation sequencing. Recent advances in genetic engineering, genotyping, high-resolution imaging, and biomarker testing have made it easier to deliver the right treatments to the right patients at the right time. This course serves to present an overview of gene-editing strategies from the leading experts who have pioneered it in other disciplines, followed by examples in eye and vision science and practical applications. Information that will be covered in this course includes variants of CRISPR and Cas proteins, guide RNA (gRNA) selection and synthesis, gene editing, RNA editing, delivery to a cell, and off-target analysis.

Bring your laptop for the interactive afternoon workshop.

Learning objectives
After attending this course, participants will be able to:

- Discuss the latest advances in genome engineering and its translational applications to various diseases
- Compare and contrast the benefits and uses of different gene-editing strategies
- Identify the best practices for selecting gRNAs that are both highly active and specific

Agenda
Presenters and presentations may change.

8 – 8:30am  Welcome and introduction to gene editing
Stephen H. Tsang, MD, PhD, Edward Harkness Eye Institute, Columbia University, New York, NY
In the last decade, the field of genome engineering has made innumerable groundbreaking advances. Amongst the most important of these technical developments are FDA-approved gene medicine supplementation therapy and novel CRISPR-Cas systems. This field is both exciting and developing at an exponential pace, spurred by the promise of generating precise modifications in the genome for not only discovery science but also potential clinical applications. Breakthroughs in gene delivery vectors, both viral and non-viral, have enabled the delivery of genetic payloads in preclinical models of retinal disorders and have paved the way for numerous successful clinical trials. Moreover, the adaptation of CRISPR-Cas systems for genome surgery has led to the correction of both recessive and dominant pathogenic alleles, expanding the disease-modifying power of gene therapies. This presentation will provide an introductory overview of the mechanisms underlying different gene editing strategies and their promising applications in human disease.

8:30 – 9am  Genome engineering of human iPS and mouse embryo
Andras Nagy, PhD, Lunenfeld-Tanenbaum, Research Institute, Mount Sinai Hospital, New York, NY
The concerns of cell therapy safety and the unsolved need for long-term allogeneic cell acceptance without immunosuppression withhold cell-based therapies from broad clinical applications. Recently, we established two platform technologies that can act synergistically to address those major hurdles. First, the safe-cell system was meticulously designed to mitigate the risk of tumour formation from therapeutic cells. Second, we have developed a method that allows for long-term allogeneic cell acceptance without immune suppression of the recipients.

In this presentation, we demonstrate that cells exhibiting these two platform technologies can be genetically engineered to secrete therapeutic biologics in a controlled manner following delivery to the blinding eye. We have derived eye resident cells that can act as factories for various local acting biologics developed in our lab. Furthermore, the cell type of these therapeutic cells and the biologics they secrete are relevant to the degenerating retina and we are currently testing their therapeutic effects in vitro and in vivo.

Note: I would like to acknowledge the co-authorship of my trainees: Dr. Sabiha Hacibekiroglu, Eric Jong, Dr. Kristina Nagy, and Dr. Jeffrey Harding.

9 – 9:30am  
**Genome engineering delivery system**  
*Krishanu Saha, PhD, Associate Professor, Department of Biomedical Engineering & Wisconsin Institute for Discovery, University of Wisconsin-Madison, Madison, WI*  
Genome editing holds promise in disrupting or correcting pathogenic mutations affecting the eye, and these strategies are now being tested in clinical trials. Genome editors can directly modify endogenous genes in contrast to traditional gene augmentation therapy, but delivering genome-editing machinery to the right cells in the retina and other structures of the eye is a key challenge in the field. Here I will discuss delivery strategies that target various cell types in the eye. Both the viral and nonviral strategies will be presented, as well as different types of cargoes within these delivery vectors, employing CRISPR-Cas9, base editors, etc. Bottlenecks in performing genome editing with high precision and accuracy will be presented. An emerging set of genome editing tools being developed by the NIH Somatic Cell Genome Editing Consortium will finally be highlighted.

9:30 – 10am  
**Prime-, Base-, Editing and Cas13a RNA editing**  
*Katherine Wert, PhD, Assistant Professor of Ophthalmology & Molecular Biology at UT Southwestern Medical Center, Dallas, TX*  
Genome engineering through the CRISPR (clustered regularly interspaced short palindromic repeats)/Cas9 system has revolutionized technology in many fields. However, CRISPR gene editing also has its limitations for precision gene editing as a therapeutic strategy. Precise gene editing using homology-directed repair (HDR) requires the presence of a homologous DNA template to guide the specific repair process. Non-homologous end joining (NHEJ), a second cellular DNA repair pathway, occurs at a higher rate than HDR in eukaryotic cells, and especially within non-dividing cells. CRISPR base editing is a new method of genome editing that allows for the conversion of a specific DNA base into another base at a targeted genomic locus, without using double-stranded breaks of the DNA as needed in traditional CRISPR/Cas9 methods. This presentation will discuss the methodology and mechanism of CRISPR base-editing for research and therapeutic applications. Additionally, this presentation will cover the use of CRISPR technology to cut RNA, instead of DNA, with the use of Cas13. The ability to edit RNA with Cas13 allows for the possibility of treating human disease without directly altering the genome.
10 – 10:15am  
**Morning Break**

10:15 – 10:45am  
**CRISPR/Cas Base Editing ... steps towards direct mutation correction for monogenic eye disease**

*Alex W. Hewitt, PhD, Professor in Ophthalmology, Menzies Institute for Medical Research, University of Tasmania, Tasmania, Australia*

This presentation will provide an overview of the current status of CRISPR/Cas base editing, where a nuclease dead or disabled Cas9 is used to direct an enzyme to deaminase a specific nucleotide. Base editing circumvents potential risks associated with introducing double strand breaks into the genome, which can lead to imprecise insertions and deletions (indels), and has been found to have a dramatically higher editing efficiency compared to homology-directed repair (HDR).

10:45 – 11:15am  
**Gene therapy in the cornea**

*Tara C. B. Moore, PhD, Professor, School of Biomedical Science, Ulster University, Ulster, Northern Ireland, UK*

Gene therapy for inherited corneal dystrophies and recent progress with CRISPR and siRNA will be discussed.

We have previously presented our work investigating the extensive design and testing of corneal dystrophy mutation-specific siRNA and CRISPR guides required to maximise on-target and minimise off-target effects and shown proof-of-concept for both our approaches both *in vitro* and in a patient-derived cell line. Application of these technologies *in vitro* by delivery of siRNA or CRISPR components to the cornea by non-viral, topically administered delivery methods will be discussed.

TGFBI corneal dystrophy is often induced or exacerbated by corneal injury in man. Animal models of R124H GCD2 (Avellino) TGFBI corneal dystrophy spontaneously develop corneal opacities at a much lower frequency and at a much older age than humans. Rapid development of corneal opacity following corneal injury in a mouse model will be discussed together with *in vitro* functionality of siRNA and CRISPR gene editing in the prevention of TGFBI corneal dystrophy progression.

11:15 – 11:45am  
**CRISPR-based genome editing for neovascular age-related macular degeneration**

*Glenn Yiu, MD, PhD, Associate Professor, University of California at Davis, Davis, CA*

This presentation will discuss current efforts using CRISPR-based genome editing approaches to target angiogenic factors such as vascular endothelial growth factor (VEGF) for suppression of choroidal neovascularization (CNV) for the treatment of neovascular age-related macular degeneration (nAMD). In particularly, we will discuss the use of different modes of Cas9 endonuclease delivery such as adeno-associated viral (AAV) vectors, different Cas9 orthologs, gRNA selection and strategy, methods to measure gene editing efficiency in vivo, methods to assess functional efficacy for suppressing CNV, and assays to detect off-target activity. We previously reported the successful use of lentiviral vectors to express SpCas9 to suppress VEGF secretion from the human RPE cell line ARPE19. We have since demonstrated that AAV vectors can effectively deliver SpCas9 and SaCas9 to mouse eyes in vivo, and that SpCas9 delivered with gRNAs using dual AAV vectors was more effective than SaCas9 expressed with gRNAs as single AAV vectors to suppress VEGF and laser-induced CNV. We also found that inclusion of double gRNAs is more effective than single gRNAs for
gene ablation, and that next-generation sequencing (NGS) provides a more robust quantification of gene editing activity than traditional methods such as the T7E1 mismatch detection assay or Tracking of Indels by Decomposition (TIDE). Finally, we describe various modes of quantifying laser-induced CNV as well as both candidate-based and non-biased methods of detecting off-target activity. Our findings provide a framework for deploying genome editing as a therapeutic approach for one of the leading causes of vision loss in the elderly.

11:45 – 12pm  **CRISPR based point-of-care molecular diagnostics: DETECTR (DNA endonuclease targeted CRISPR Trans Reporter)**  
*Peter Quinn, PhD, Fellow, Columbia University, New York, NY*  
The ever-expanding utility of the CRISPR-Cas systems is moving us closer to point-of-care (POC) molecular diagnostics. Crucial considerations for the detection of nucleic acids include the specificity, sensitivity and speed of the method utilized. This presentation will explore novel CRISPR-Cas9 systems, compare their strengths and weaknesses, and consider their practical applications. The DETECTR systems, developed by the Doudna lab, rely on the target-activated non-specific single-stranded DNA (ssDNA) cleavage ability of the Cas proteins. When paired with an ssDNA fluorescent reporter system, this technique allows for attomolar sensitivity of nucleic acids. The Cas12a- and Cas14-DETECTR systems have been utilized for the detection of human papillomavirus (HPV) in patient samples and the identification of single nucleotide polymorphisms (SNPs) in the HERC2 gene, respectively. DETECTR along with Specific High Sensitivity Enzymatic Reporter UnLOCKING (SHERLOCK; which utilize Cas13) and Cas12b-mediated DNA detection (CDetection) provide a set of tools that can propel POC molecular diagnostics rapidly forward.

12 – 12:15pm  **Morning wrap-up and summary/questions**  
*Aliaa Abdelhakim, MD, PhD, Applied Genetics Clinic New York Presbyterian Hospital, New York, NY*

12:15 – 1:15pm  **Lunch**

1:15 – 1:30pm  **Off-Targeting, GMP production and FDA**  
*Vinit B. Mahajan, MD, PhD, Vice Chairman, Stanford University, Palo Alto, CA*  
Recent concerns regarding the clinical utilization of clustered regularly interspaced short palindromic repeats (CRISPR) involve uncertainties about the potential detrimental effects that many arise due to unintended genetic changes, as in off-target mutagenesis, during CRISPR genome surgery. This lecture will give an overview of off-targeting detection methods and CRISPR’s place in the clinical setting, specifically in the field of ophthalmology.

As CRISPR utilization in the laboratory setting has increased, knowledge regarding CRISPR mechanisms including its off-target effects has also increased. Although a perfect method for achieving 100% specificity is yet to be determined, the past few years have seen many developments in off-targeting detection and in increasing efficacy of CRISPR tools. The CRISPR system has high potential to be an invaluable therapeutic tool as it has the ability to modify and repair pathogenic retinal lesions. Although it is not yet a perfect system, with further efforts to improve its specificity and efficacy along with careful screening of off-target mutations, CRISPR-mediated genome surgery potential can become maximized and applied to patients.
Identifying drug targets using CRISPR-mediated functional genomics

Falak Sher, PhD, Assistant Professor of Neurological Sciences, Columbia University, New York, NY

This lecture will provide an overview of the CRISPR-mediated functional genomics that can be used to identify potential drug targets within protein complexes and regulatory genomic region.

Continuing medical education agenda ends. The following sessions are not included in the CME program.

Improved methods for efficient CRISPR-based genome engineering in iPSCs

Kevin Holden, PhD, Head of Science, Synthego Corporation

Specific genetic alterations can be engineered into induced pluripotent stem cells (iPSCs) using technologies such as CRISPR in order to generate more approximate models of human disease than immortalized cell lines. Additionally, there is great promise in utilizing iPSCs as cellular therapies for genetic disease.

However, the efficient engineering of iPSCs, even with the advent of CRISPR, remains a challenge. We will demonstrate some improved methods for the engineering of gene knockouts, single nucleotide variant knock-ins and gene tag insertions using a CRISPR strategy that relies on the delivery of an RNP format using chemically modified, synthetic sgRNA and single-stranded donor DNA templates of optimal design.

These methods make it possible to perform functional gene knockouts and targeted knock-ins at very high rates, sometimes above 90% in a cell population. Such efficiencies lead to the rapid identification and expansion of edited iPSC clones for downstream applications, and allow for the development of a high-throughput platform for iPSC engineering.

Mutant Cas9/Cas12a enzymes that are highly specific, efficient, and demonstrate superior HDR

Michael Collingwood, Integrated DNA Technologies

While the SpCas9 and AsCas12a nucleases are attractive options to do genome editing, these bacterial proteins are poorly adapted to function specifically and efficiently in eukaryotic cells. To address these shortcomings, we used directed evolution in bacteria to select for a more specific SpCas9 mutant (HiFi Cas9) and a more potent AsCas12a mutant (Cas12a Ultra). These mutant proteins improve the overall quality of genome editing when delivered into cells as ribonucleoprotein (RNP) complexes. Efficient and specific CRISPR nucleases are a critical part of IDT’s complete CRISPR workflow, which includes design optimizations for guide selection, best-in-class gRNAs, optimized CRISPR enzymes, and homology-dependent repair reagents and protocols.

Afternoon break
2:30 – 4pm  Guide RNA Design Workshop

Nan-Kai Wang, MD, PhD, Professor, Chang Gung Memorial Hospital, Tao-Yuan, Taiwan, ROC, Joseph Ryu, Columbia University, New York, NY, and Sarah Levi, Columbia University, New York, NY

Current FDA-approved gene therapy trials primarily focus on the treatment of autosomal recessive diseases through gene supplementation. However, treating autosomal dominant (ad) disorders require the removal of a gain-of-function allele and therefore remains a challenge. This presentation will provide a comprehensive overview of the novel knockout homologous-directed repair (HDR) strategy and its therapeutic applications in ophthalmology including Best1 Vitelliform Macular Dystrophy (VMD), an inherited retinal dystrophy caused by ad mutations. Following a Guide RNA design demonstration, the audience will have the chance to practically apply these principles in a hands-on workshop where they design their own Guide RNAs.

4 – 4:30pm  Afternoon wrap up and summary/ questions with discussion panel

Vinit B. Mahajan, MD, PhD, Vice Chairman, Stanford University, Palo Alto, CA