Workshop on Genome Editing Tool Developments and Clinical Applications

3 April 2025, Hong Kong

Acknowledgements

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Programme

Date: **April 3, 2025 (Thursday)**

Venue: **INNO2 Multi-Function Room 2-3, 2/F, Building 17W, Hong Kong Science Park**

Time	Agenda Item	Speaker	Moderator
08:30 - 09:00	**Registration (1/F Lobby, Building 17W)**		
09:00 - 09:05	**Welcoming remarks** Mr. Albert Wong CEO, Hong Kong Science and Technology Parks Corporation		**Dr. Cynthia Shum,** Senior Scientist, GenEditBio
09:05 - 09:15	**Opening remarks** **Prof. Sandra Ceccatelli, MD, PhD** Professor, Department of Neuroscience, Karolinska Institutet, Sweden; Director, Ming Wai Lau Centre of Reparative Medicine, Karolinska Institutet, Sweden **Prof. Yu Huang, PhD** Chair Professor of Biomedical Sciences and Vascular Biology; Jeanie Hu Professorship in Biomedical Sciences; Head, Department of Biomedical Sciences, City University of Hong Kong		
09:15 – 09:45	**Engineering approaches to improve the precision of genome editing** **Prof. Ben P. Kleinstiver, PhD** Associate Professor, Center for Genomic Medicine, Harvard Medical School & Massachusetts General Hospital, Boston, USA; Kayden-Lambert MGH Research Scholar		
09:45 - 10:15	**From Gene Editing to New Frontiers: Advancing Cell and Gene Therapies** **Prof. Wensheng Wei, PhD** Professor, School of Life Sciences, Peking University, China Director, Genome Editing Research Centre, Peking University, China		
10:15 - 10:45	**The Landscape of Genetic Diseases in Saudi Arabia: Challenges and Opportunities for Gene Therapy** **Prof. Aiman Alhazmi, PhD** Assistant Professor of Human Genetics Dean, College of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, the Kingdom of Saudi Arabia		
10:45 - 11:00	Coffee & tea break; networking		
11:00 - 11:30	**Prospects for Gene and Epigenome Editing in Hepatitis B Disease** **Prof. Man Fung Yuen, DSc, MD, PhD, MBBS** Chair Professor of Gastroenterology and Hepatology; Li Shu Fan Medical Foundation Professor in Medicine, The University of Hong Kong		**Prof. Bo Feng**
11:30 - 12:00	**Precise genome editing and the translational studies** **Prof. Dali Li, PhD** Professor of Biochemistry and Molecular Biology, School of Life Sciences, East China Normal University; Scientific Director, Gene Editing Technology, China Pharmaceutical Biotechnology Association		
12:00 - 12:30	**Exploring RNA machineries for DNA manipulation** **Prof. Junjie Liu, PhD** Associate Professor, School of Life Science, Tsinghua University, China		
12:30 - 14:00	Lunch		
14:00 - 14:25	**In Vivo CRISPR Gene Editing in Patients with Herpes Stromal Keratitis: from phase 0 to phase 1 study** **Prof. Jiaxu Hong, MD, PhD** Professor, Eye & ENT Hospital of Fudan University, Shanghai, China		**Prof. Wenjun Xiong**
14:25 - 14:50	**High-throughput engineering of CRISPR tools with combinatorial mutagenesis and machine learning** **Prof. Alan Siu Lun Wong, PhD** Associate Professor, School of Biomedical Sciences, The University of Hong Kong		

Time	Agenda Item	Speaker	Moderator
14:50 - 15:15	**AAV-CRISPR-mediated liver-specific knock-in restored hemostasis in neonatal hemophilia B mice** **Prof. Bo Feng, PhD** Associate Professor, School of Biomedical Sciences, The Chinese University of Hong Kong		
15:15 - 15:25	Coffee & tea break; networking		
15:25 – 15:50	**Lipid nanoparticle-CRISPR mediates gene editing in trabecular meshwork and induces ocular hypertension in mice** **Prof. Wenjun Xiong, PhD** Associate Professor, Department of Biomedical Sciences, City University of Hong Kong		**Prof. Alan Siu Lun Wong**
15:50 - 16:15	**Therapeutic genome editing for neurological diseases** **Prof. Jae Young Lee, PhD** Assistant Professor, Ajou University School of Medicine, Suwon, Korea		
16:15 – 16:40	**Development of in vivo genome editing strategies for treating familial Alzheimer's disease** **Prof. Yangyang DUAN, PhD** Research Assistant Professor, Division of Life Science, The Hong Kong University of Science and Technology		
16:40 - 16:55	**Machine-learning guided low-N search for top variants for genome editor engineering** **Dr. Athena CHU** (Prof. Alan Wong's lab), The University of Hong Kong		
16:55 - 17:10	**A Comparative Study of CRISPR-Based In vivo Gene Knock-In Strategies for Haemophilia B Treatment and Editing Outcomes in Genome** **Ms. Siqi Zhang** (Prof. Bo Feng's lab), The Chinese University of Hong Kong		**Prof. Zongli Zheng**
17:10 - 17:25	**Mutation-independent gene knock-in therapy targeting 5'UTR for autosomal dominant retinitis pigmentosa** **Mr. Baoshan Liao** (Prof. Wenjun Xiong's lab), City University of Hong Kong		
17:25 - 17:40	**GenomePAM directs PAM characterization and engineering of CRISPR-Cas nucleases** **Mr. Miao Yu** (Prof. Zongli Zheng's lab), City University of Hong Kong		
17:40 - 17:55	**Enhancing Gene Editing Precision: The Role of RhampSeq™ CRISPR System for Safer Cell Therapy** **Dr. Edward Wong Sern Yuen, PhD** Senior Manager of Gene Writing and Editing, APAC, Integrated DNA Technologies (IDT)		
17:55 – 18:00	**Concluding remarks** **Prof. Zongli Zheng, PhD** Associate Professor, Dept. Biomedical Sciences, City University of Hong Kong Principal Investigator, Karolinska Institutet (InnoHK)		

Guest Speakers

Biography & Abstract

The Landscape of Genetic Diseases in Saudi Arabia: Challenges and Opportunities for Gene Therapy

Prof. Aiman Alhazmi, PhD

Assistant Professor of Human Genetics; Dean, College of Science and Health Professions, King Saud bin Abdulaziz University for Health Sciences, Riyadh, The Kingdom of Saudi Arabia

Biography

Dr. Aiman Alhazmi is the Dean of the College of Science and Health Professions at King Saud bin Abdulaziz University for Health Sciences (KSAU-HS), appointed in January 2025. He earned his bachelor's degree in clinical laboratory sciences in Saudi Arabia before moving to the United States, where he completed his Master's and Ph.D. in Human Genetics at Virginia Commonwealth University. His Ph.D. research focused on understanding the role of nucleosome remodeling complexes in regulating gene expression in mouse embryonic stem cells.

He joined King Saud bin Abdulaziz University for Health Sciences (KSAU) in late 2015 as an Assistant Professor of Human and Molecular Genetics. In 2019, he took on a clinical role in the Department of Pathology and Laboratory Medicine as a Clinical Scientist in the Molecular Genetics section, where he was actively involved in genetic diagnostics.

His research focuses on investigating the genetic basis of inherited diseases, with a particular interest in understanding the molecular mechanisms underlying genetic variants associated with rare and common disorders in Saudi Arabia. Through his work, he aims to bridge clinical genetics with genomic medicine, contributing to the development of precision medicine and genomic therapeutics in the region.

Abstract

Rare genetic diseases present significant clinical and public health challenges, particularly in populations with high consanguinity rates, such as Saudi Arabia. This talk will provide an overview of the rare disease landscape in Saudi Arabia, highlighting the most prevalent genetic disorders and shedding light on opportunities for establishing genomic therapeutics and collaborations with international partners.

Development of in vivo genome editing strategies for treating familial Alzheimer's disease

Prof. Yangyang DUAN, PhD

Research Assistant Professor, Division of Life Science, The Hong Kong University of Science and Technology

Biography

Dr. Yangyang DUAN is a Research Assistant Professor at the Division of Life Science, State Key Laboratory of Molecular Neuroscience and Daniel and Mayce Yu Molecular Neuroscience Center, The Hong Kong University of Science and Technology. Her research focuses on pioneering genome-editing strategies to address neurodegenerative disorders such as Alzheimer's disease. She has developed the first non-invasive brain-wide genome-editing system and demonstrated its therapeutic potential for treating familial Alzheimer's disease. This technology was recognized by the Chinese Neuroscience Society as one of the Major Neuroscience Breakthroughs of 2021, the first of its kind in the Greater Bay Area. Dr. DUAN has published her research in high-impact journals such as Nature Biomedical Engineering, Nature Neuroscience, and Proceedings of the National Academy of Sciences. Her work has introduced new perspectives for tackling complex neurological disorders and was honored with two Gold Medals at the 49th International Exhibition of Inventions of Geneva, Switzerland.

Abstract

Familial Alzheimer's disease (AD) is caused by dominant mutations in the genes encoding amyloid precursor protein (APP), presenilin 1, and presenilin 2, which lead to overproduction and pathological accumulation of amyloid-beta across various brain regions. CRISPR/Cas9-mediated genome editing has emerged as a promising therapeutic approach for targeting these disease-causing mutations and modifying disease progression. However, developing effective genome editing strategies for treating familial AD requires achieving efficient and widespread editing across the multiple brain regions affected by AD pathology. In this study, we developed a brain-wide genome editing system by combining a modified adeno-associated virus capable of crossing the blood-brain barrier with CRISPR-mediated genome editing technology. We demonstrated that a single systemic delivery of this brain-wide genome editing system efficiently disrupted mutant APP across various brain regions, significantly reducing amyloid plaque deposition throughout the brain in transgenic mouse models carrying familial AD mutations. This treatment also alleviated key AD-associated pathologies, including microgliosis and neurite dystrophy. Behavioral assessments showed that the brain-wide genome editing system ameliorated cognitive deficits in AD transgenic mice. Notably, the beneficial effects of this brain-wide genome editing system persisted for over two years. These findings underscore the potential of CRISPR/Cas9-mediated genome editing as a single-administration, disease-modifying treatment for familial AD. Moreover, this approach could be extended to other monogenic diseases affecting multiple brain regions, offering a promising avenue for the development of targeted therapies for neurological disorders.

AAV-CRISPR-mediated liver-specific knock-in restored hemostasis in neonatal hemophilia B mice

Prof. Bo Feng, PhD

Associate Professor, School of Biomedical Sciences, The Chinese University of Hong Kong

Biography

Prof. FENG Bo (馮波), Associate Professor in the School Biomedical Sciences, Faculty of Medicine, The Chinese University of Hong Kong. Prof. Feng graduated from Nankai University with B.Sc. (1993) and M.Sc (1996), and received her Ph.D. (2006) from National University of Singapore. After graduation, Prof. Feng joined Prof. Ng Huck Hui's lab in Genome Institute of Singapore as a postdoc. She worked on stem cells and reprogramming and published her works in Nature Cell Biology, Cell Stem Cell and Nature. In Nov 2010, Prof. Feng joined CUHK, and is an active member in the Developmental and Regenerative Biology Thematic Research Program, Institute for Tissue Engineering and Regenerative Medicine, and CUHK-GIBH Joint Laboratory. Her current research interest lies within the molecular mechanism that controls pluripotency and differentiation of ESCs/iPSCs, as well as development of new strategies based on CRISPR technology for gene and cell therapy.

Abstract

AAV-delivered CRISPR/Cas9 (AAV-CRISPR) has shown promising potentials in preclinical models to efficiently insert therapeutic gene sequences in somatic tissues. However, the AAV input doses required were prohibitively high and posed serious risk of toxicity. Here, we performed AAV-CRISPR mediated homology-independent knock-in at a new target site in mAlb 3'UTR and demonstrated that single dose of AAVs enabled long-term integration and expression of hF9 transgene in both adult and neonatal hemophilia B mice (mF9 -/-), yielding high levels of circulating human Factor IX (hFIX) and stable hemostasis restoration during entire 48-week observation period. Furthermore, we achieved hemostasis correction with a significantly lower AAV dose through liver-specific gene knock-in using hyperactive hF9R338L variant. The plasma antibodies against Cas9 and AAV in the neonatal mice receiving low-dose AAV-CRISPR were negligible, which lent support to the development of AAV-CRISPR mediated somatic knock-in for treating inherited diseases.

In Vivo CRISPR Gene Editing in Patients with Herpes Stromal Keratitis: from phase 0 to phase 1 study

Prof. Jiaxu Hong, MD, PhD

Professor, Eye & ENT Hospital of Fudan University

Biography

2011 Graduated from Fudan University

2011-2014 Resident, Eye and ENT Hospital of Fudan University

2014-2017 Attending Physician, Eye and ENT Hospital of Fudan University

2017-2022 Associate Chief Physician, Eye and ENT Hospital of Fudan University

2022-Present Senior Scientist, Eye and ENT Hospital of Fudan University

2022-Present Chief Physician, Eye and ENT Hospital of Fudan University

2023-Present Professor, Department of Ophthalmology, Fudan University

Starting with the innovation and optimization of gene therapy strategies, and characterized by the integration of medicine and engineering, we conduct a series of innovative research and translational studies on biological therapies for corneal diseases.

Abstract

In vivo CRISPR gene therapy holds large clinical potential, but the safety and efficacy remain largely unknown. Here, we injected a single dose of herpes simplex virus 1 (HSV-1)-targeting CRISPR formulation in the cornea of three patients with severe refractory herpetic stromal keratitis (HSK) during corneal transplantation. Our study is an investigator-initiated, open-label, single-arm, non-randomized interventional trial at a single center (NCT04560790). We found neither detectable CRISPR-induced off-target cleavages by GUIDE-seq nor systemic adverse events for 18 months on average in all three patients. The HSV-1 remained undetectable during the study. Our preliminary clinical results suggest that in vivo gene editing targeting the HSV-1 genome holds acceptable safety as a potential therapy for HSK. In this report, we will present the data obtained from phase 0 to phase 1.

Engineering approaches to improve the precision of genome editing

Prof. Ben P. Kleinstiver, PhD

Associate Professor, Center for Genomic Medicine, Harvard Medical School & Mass General Hospital

Kayden-Lambert MGH Research Scholar

Biography

Dr. Kleinstiver is an Associate Professor in the Center for Genomic Medicine and Department of Pathology at Massachusetts General Hospital and Harvard Medical School, and is the Kayden-Lambert MGH Research Scholar 2023-2028. He is a biochemist by training and is now a 'genome editor' whose research seeks to modify genetic material by optimizing new methods to engineer new genome editing technologies. Some of the major goals in the Kleinstiver lab are to develop safe, effective, and versatile tools that can correct genetic mutations that underlie human disease.

Abstract

Methods to engineer proteins have been deployed to enhance the intrinsic properties of CRISPR-Cas genome editing enzymes and have facilitated the development new editing technologies. However, most approaches to characterize the resultant molecules are laborious and do not adequately scale, leading to blind spots in engineering trajectories or an inability to achieve maximally efficient and precise genome editing. We are therefore developing methods to more scalably investigate the biochemical properties of genome editing technologies, permitting the optimization of a large suite of new tools. In one instance, we utilized bacterial selections, scalable characterization methods, and machine learning to engineer a more complete toolbox of bespoke CRISPR-Cas proteins that permits researchers to select an enzyme with properties tuned to a particular target site, offering advantages in terms of efficiency and safety. Separately, we are engineering other genome editing technologies that leverage the advantageous properties of DNA-dependent DNA polymerases (DDPs) to install short, medium, or large sized DNA edits. Click editors utilize simple oligonucleotides tethered to a Cas9-DDP-bound target site as a template for genome writing, permitting the installation of edits encoded on the template DNA molecule. Together, these engineered enzymes offer new capabilities for precision genome editing to generate small and larger genetics edits, simplifying editing strategies towards the development of new tools and genomic medicines.

Therapeutic genome editing for neurological diseases

Prof. Jae Young Lee, PhD

Assistant Professor, Ajou University School of Medicine, Suwon, Korea

Biography

Jae young Lee is an assistant professor of Medicine at Ajou University, South Korea. Jae received a B.Eng (Hons) from University of Melbourne and a PhD in Neuroscience from Monash University (Central Clinical School). He was previously the managing director of strategy & business development at ToolGen, a KOSDAQ listed biotech company based in Seoul, South Korea focusing on translating CRISPR/Cas9 gene editing technology to novel therapeutics. His research studies understanding and targeting diseases at genetic level.

Abstract

Genome editing holds tremendous promise for the development of innovative therapies. Because of its versatility and ability to edit the genetic cause, CRISPR/Cas9-based genome editing has the potential to treat or potentially cure intractable diseases. Genome editing can also be applied for neurological diseases where current therapeutic modalities have difficulties in delivering therapeutic components to the nervous system. Adeno-associated virus (AAV) has long been utilized as gene therapy vehicle especially for brain disorders, due to its favourable safety profile and neurotrophic nature. Throughout this seminar, I will discuss the potential of AAV-mediated gene editing for the treatment of neurological diseases especially diseases where therapeutic strategy involves targeting glial cells.

Precise genome editing and the translational studies

Prof. Dali Li, PhD

Professor of Biochemistry and Molecular Biology,
School of Life Sciences, East China Normal University

Scientific Director, Gene Editing Technology, China Pharmaceutical Biotechnology Association

Biography

Dali Li is a professor of biochemistry and molecular biology in the School of Life Sciences at East China Normal University. He is also the director of the Shanghai Frontiers Science Center for Genome Editing and Cell Therapy. From 1997 to 2007, Dali Li studied at the School of Life Sciences at Hunan Normal University, where he received his B.S. and Ph.D. degrees. Between 2004 and 2007, he worked as a visiting scholar at the Albert B. Alkek Institute of Biosciences and Technology at Texas A&M University Health Science Center. In 2007, he joined East China Normal University as a Lecturer and was promoted to Full Professor in 2014. His research focuses on developing genome editing technologies and the applications of the CRISPR/Cas system for the treatment of genetic disorders. His group was among the first to report the generation of genetically modified mouse and rat disease models using TALEN and CRISPR/Cas technology, and they have developed several innovative base editors, including hyperactive cytosine base editors (hyCBEs), dual base editors (A&C-BEmax), ABE with minimal bystander editing (ABE9), and a series of TadA-derived CGBE/CBEs (Td-CGBE/CBEs), adenosine transversion base editors (AXBE and ACBEs), highly accurate cytosine base editors (haCBEs) and IscB-based efficient miniature and efficient editing tools. Leveraging advancements in Cas9 technology, in collaboration with colleagues, his group has successfully devised strategies to ameliorate several genetic diseases in animal models and demonstrated the clinical application of Cas9 technology in the treatment of β-thalassemia patients.

Abstract

To date, approximately 95% of genetic disorders have no approved treatments. Gene therapy is a promising strategy for certain hereditary diseases, but current strategies possess some disadvantages, such as a short duration of exogenous gene expression or tumorigenic potential. With the emergence of genome editing technology, particularly the CRISPR/Cas system, efficient, site-specific genome modification has become practicable. In this talk, I will present our recent published and unpublished studies of highly efficient and accurate genome editing technologies, such as several improved base editors and genome editing based gene therapies.

Exploring RNA machineries for DNA manipulation

Prof. Junjie Liu, PhD

Associate Professor, School of Life Science, Tsinghua University, Beijing, China

Biography

Jun-Jie Gogo Liu received his Ph.D. from the School of Life Sciences at Tsinghua University in 2016. He completed postdoctoral training at Lawrence Berkeley National Laboratory and UC Berkeley, and was a Life Science Research Fund Fellow sponsored by Pfizer. He joined Tsinghua University as an Assistant Professor in 2020 and was promoted to Associate Professor in 2023. His research focuses on the design and development of new gene-editing tools and the study of RNA-involved nuclease machinery.

Abstract

RNA, as a critical biological macromolecule, plays a pivotal role in various biological processes by regulating or guiding the functions of proteins and even directly catalyzing biological reactions. In practical applications, RNA elements are generally more programmable and maneuverable than proteins, offering new dimensions and possibilities for functional carriers of biotechnology. In my presentation, I will discuss our focus on RNA elements to discover new bio-machineries such as CRISPR-Cas, transposons, ribozymes, etc., and employ biochemical and structural tools to comprehend their mechanisms of action for potential applications in biomolecular manipulation like gene editing. Furthermore, through systematic characterization of RNA-associated bio-machineries, we have observed trends in the functional succession between RNA and protein across multiple systems, providing valuable insights into the molecular evolution of life.

From Gene Editing to New Frontiers: Advancing Cell and Gene Therapies

Prof. Wensheng Wei, PhD

Professor, School of Life Sciences; Director, Genome Editing Centre, Peking University, Beijing, China

Biography

Wensheng Wei received his bachelor degree in Biochemistry from Peking University, Ph.D. in Genetics from Michigan State University. After postdoctoral training and working as a research associate at Stanford University School of Medicine, Dr. Wei became a principle investigator in the School of Life Sciences at Peking University from 2007. He is currently a professor at the Biomedical Pioneering Innovation Center, Peking-Tsinghua Center for Life Sciences, and the School of Life Sciences at Peking University, Director of the Genome Editing Research Center at Peking University, and Lead Scientist at Changping Laboratory. The Wei group's research primarily focuses on advancing eukaryotic gene editing tools, with particular emphasis on high-throughput functional genomics, gene and cell therapy, and innovative RNA therapeutics utilizing circular RNAs.

Abstract

This presentation explores advancements in cellular and RNA-based therapies, with a focus on allogeneic CAR-T therapy, γδ T cell function enhancement, and RNA-based treatments. Achieving allogeneic CAR-T therapy has been difficult due to immuno-rejection and lack of persistence. Our research using genome-wide CRISPR screens identified specific genetic modifications in allogeneic T cells that reduce immune clearance by multiple effectors. The first-in-human anti-CD19 allogeneic CAR-T therapy in a clinical trial has shown a 100% response rate and effective tumor control. Additionally, we found that certain genetic alterations in human Vγ9Vδ2 T cells enhance cytotoxicity and persistence, driving their transition towards an NK cell-like phenotype, enabling effective leukemia cell killing. In RNA-based therapies, the LEAPER 2.0 system for efficient RNA editing has been validated in a non-human primate model, showing significant improvements in motor abilities with lasting effects on Duchenne muscular dystrophy. In mitochondrial gene editing, we introduce mitochondrial DNA base editors (mitoBEs), which combine transcription activator-like effector (TALE)-fused nickase and a deaminase for precise base editing in mitochondrial DNA. Engineered mitoBEs optimized to reduce off-target effects were used to develop mouse models of mitochondrial diseases, displaying phenotypes corresponding to human conditions like Leigh disease and LHON. The edited mitochondrial DNA persisted across tissues and was maternally inherited, highlighting the potential for lasting therapeutic impact. These innovations collectively hold substantial promise for precision medicine, therapeutic RNA editing, and organelle gene editing.

High-throughput engineering of CRISPR tools with combinatorial mutagenesis and machine learning

Prof. Alan Siu Lun Wong, PhD

Associate Professor, School of Biomedical Sciences, The University of Hong Kong

Biography

Dr. Alan Siu-lun Wong is an Associate Professor at School of Biomedical Sciences of The University of Hong Kong (HKU). His research takes an integrative approach leveraging on techniques in synthetic biology, CRISPR-based genome editing, combinatorial genetics, and high-throughput functional genomics to decode the complex genetics of human diseases, as well as engineer gene editing and cellular tools for providing new biomedical and biotechnological solutions. His work has resulted in publications in prestigious journals including Nature Methods, Nature Biomedical Engineering, Nature Biotechnology, Nature Cell Biology, Nature Neuroscience, Nucleic Acids Research, Cancer Research, PNAS, Cell Systems, Cell Reports, as well as PCT patents and patent applications on CRISPR-based screening methods and tools. He was awarded the Croucher Foundation Studentship (2008), Butterfield-Croucher Award (2008), Croucher Foundation Fellowship (2012), Hong Kong Institution of Science Young Scientist Award in Life Science (2011), RGC Early Career Award (2016), NSFC Excellent Young Scientists Award (Hong Kong and Macau) (2020), HKU Outstanding Young Researcher Award (2023), and the BOCHK Science and Technology Innovation Prize in Life and Health (2023).

Abstract

The combined effect of multiple mutations on protein function is hard to predict, thus the ability to functionally assess a vast number of protein sequence variants would be enormously useful for protein engineering. We have developed high-throughput platforms to enable scalable assembly and parallel characterization of barcoded protein variants with high-order combinatorial modifications. We illustrate this platform, CombiSEAL, by systematically profiling a library of combination mutants of the widely used CRISPR-Cas9 nucleases to optimize its genome-editing activity in human cells. The ease of pool-assessing editing activities of SpCas9s at multiple on- and off- target sites accelerates the identification of optimized variants and facilitates the study of mutational epistasis. We further establish a machine learning-coupled combinatorial mutagenesis approach to reduce the experimental screening burden by as high as 90%. With our platforms, we have successfully identified Cas9 variants that possesses enhanced editing specificity without sacrificing potency. These platforms are readily applicable for engineering genome editors and other proteins through experimenting combinatorial modifications en mass.

Enhancing Gene Editing Precision_The Role of RhampSeq™ CRISPR System for Safer Cell Therapy

Dr. Edward WONG Sern Yuen

Senior Manager of Gene Writing and Editing, APAC, Integrated DNA Technologies (IDT)

Biography

Edward is a Senior Manager of Gene Writing and Editing at the Integrated DNA Technologies (IDT), specializing in CRISPR, Functional Genomics and Synthetic Biology solutions across the APAC region. With extensive experience in gene editing technologies, Edward plays a key role in supporting researchers and biotech companies by providing strategic insights, technical expertise, and innovation solutions for genome engineering.

At IDT, Edward collaborates closely with biopharma, CROs, and academic institutions to optimize gene synthesis and CRISPR workflows. He has been instrumental in developing business strategies that align with regional market demands, ensuring researchers have access to cutting-edge tools for drug discovery, cell and gene therapy, and functional genomics.

Additionally, Edward has a strong track record of customer engagement, scientific consultation, and market development. He actively gathers insights on emerging trends and industry needs, driving IDT's product advancements and service enhancements in CRISPR and Synthetic Biology. His expertise extends to navigating challenges such as off-target effects, precision editing, and optimizing gene synthesis for complex applications.

Abstract

CRISPR genome editing has transformed biomedical research and therapeutic development, but concerns over off-target effects remain a critical challenge. Accurately identifying and validating unintended edits is essential to ensure the precision and safety of genome editing applications. In this presentation, we introduce IDT's RhAmpSeq sequencing technology, a highly specific and scalable approach for off-target detection. RhAmpSeq leverages targeted amplicon sequencing with a unique blocking mechanism to reduce background noise, enabling researchers to confidently assess off-target events with high sensitivity.

Beyond off-target validation, we will also explore the comprehensive CRISPR solutions provided by IDT and Aldevron. IDT offers optimized guide RNA designs, high-fidelity Cas nucleases, and advanced detection methods to improve editing efficiency and specificity. Meanwhile, Aldevron provides GMP-grade nucleases for translational and clinical applications, ensuring a seamless transition from research to therapeutic development.

By integrating these cutting-edge technologies, researchers can achieve more precise and reproducible genome editing outcomes. Attendees will gain insights into how IDT and Aldevron's solutions can enhance CRISPR workflows, supporting both basic research and translational applications.

Lipid Nanoparticle (LNP)-CRISPR Mediates Robust Gene Editing in Trabecular Meshwork and Induces Ocular Hypertension in Mice through Matrix Gla Protein (Mgp) Knockout

Prof. Wenjun Xiong, PhD

Associate Professor, Department of Biomedical Sciences, City University of Hong Kong

Biography

Dr. Wenjun Xiong is currently an associate professor in the Department of Biomedical Sciences at the City University of Hong Kong. Her primary research focuses on understanding the molecular mechanisms that can facilitate the rescue, replacement, or regeneration of retinal neurons, with the ultimate goal of developing innovative gene therapy and regenerative medicine approaches for patients with retinal degeneration. Dr. Xiong received her BSc in Life Sciences from Fudan University in 2004 and completed her PhD in Biomedical Sciences at the University of Chicago in 2010. In 2011, she joined Prof. Constance Cepko's laboratory at Harvard Medical School, where she investigated the disease mechanisms of inherited blindness and pioneered gene therapies to prolong vision in mouse models. In August 2015, she became a principal investigator in the Department of Biomedical Sciences at the City University of Hong Kong.

Abstract

Lipid nanoparticles (LNPs) have transformed mRNA-based vaccines and therapeutics, emerging as a versatile vector for gene editing due to their transient expression and larger packaging capacity. However, their potential in ocular gene editing has yet to be fully explored. This study assessed the transduction pattern, inflammation, and gene editing efficiency of LNP-mRNA in mouse eyes. Intravitreally delivered LNPs demonstrated targeted mRNA expression in the trabecular meshwork with greater efficiency and specificity than adenovirus or adeno-associated virus vectors, while causing minimal microglial activation in the retinas. LNPs co-encapsulating SpCas9 mRNA and sgRNA targeting the loxP stop codon of Rosa26-CAG-LSL-tdT reporter mice resulted in robust reporter expression. Additionally, LNP-CRISPR facilitated the efficient knockout of the Matrix Gla Protein (Mgp), a gene crucial for preventing trabecular meshwork calcification. Eyes with Mgp knockout exhibited a significant and sustained increase in intraocular pressure (IOP), elongated anterior chamber depth, and decreased Ganglion Cell Complex (GCC) thickness, making it a good model for open-angle glaucoma. Our study showed that LNP-mRNA vectors can effectively mediate gene editing in trabecular meshwork, providing a promising system for developing disease models and therapeutics for glaucoma.

Prospects for Gene and Epigenome Editing in Hepatitis B Disease

Prof. Man Fung Yuen, DSc, MD, PhD, MBBS

Chair Professor of Gastroenterology and Hepatology;
Li Shu Fan Medical Foundation Professor in Medicine,
The University of Hong Kong

Biography

Professor Yuen is now the Chair Professor of The University of Hong Kong and Li Shu Fan Medical Foundation Professor in Medicine, and the Chief of the Division of Gastroenterology and Hepatology, Queen Mary Hospital, Hong Kong. He obtained his first bachelor's degree of medicine in 1992. He further pursued his academic excellence through the achievement of obtaining three doctoral degrees including Doctor of Medicine with Sir Patrick Manson Gold Medal in 2001, Doctor of Philosophy in 2005 and Doctor of Science in 2017. Professor Yuen's research interests include prevention, natural history, serology, virology and treatment of chronic hepatitis B and C, and hepatocellular carcinoma.

He is one of the top internationally renowned researchers in the field of hepatitis B disease. He has now published more than 620 papers in world-renowned medical journals including New England Journal of Medicine, Lancet, Nature Medicine, Lancet Infectious Diseases and Lancet Oncology. He has delivered more than 360 lectures all over the world. Professor Yuen is now leading most of the international trials examining new drugs including antiviral and immunomodulatory agents for the treatment of chronic hepatitis B. He is also actively performing cutting-edge research on novel markers for hepatitis B infection and occult hepatitis B infection.

Abstract

Chronic hepatitis B infection affects approximately 254 million people in the world. Up to 30% of infected people would develop end-staged liver disease including cirrhosis and liver cancer. Once the disease chronicity is established through transforming the relaxed circular virus DNA into covalently closed circular (ccc) DNA and integration of viral genome to human genome, complete virus elimination becomes practically impossible. These two forms of virus DNAs are residing in the hepatocyte nucleus and are responsible for viral replication and viral antigenemia which is associated with host immune exhaustion. The existing therapy for CHB is only acting against the reverse transcriptional activities of the virus pre-genomic RNA and mRNAs. Gene therapy targeting the cccDNA and integrated hepatitis B virus (HBV) DNA is a potential mean to eradicate the virus, and if not, to profoundly shut down viral activity. At present, there are several upcoming epigenetic modifiers which act by methylating the CpG islands of virus genomes to knock down the HBV transcription. In mice models, a single dose of these epigenetic modifiers is associated with a prolonged suppression of cccDNA and integration DNA activities with profound suppression of the levels of HBV DNA and HBV antigen (hepatitis B surface antigen - HBsAg) leading to the status of functional cure. Gene editing using endonucleases to create a double strand breaks of virus DNA leading to cccDNA elimination and integrated DNA inactivation has also been tested. Both approaches are now actively undergoing in human phase 1 clinical trial. The structure/ sequence of the mRNAs and the nucleases are designed to target the conserved HBV sequence. Meticulous experiments to improve the specificity and hence minimize the off-target effects had been performed in different cell line models. These agents need lipid nanoparticles to deliver to the target site i.e. the hepatocytes. The goal of this gene/ epigenome editing is to achieve functional cure of the disease, which is defined as undetectable viral elements namely, HBV DNA and HBsAg in the blood and patients are free of long-term viral suppressive therapy.

Postdoc/Student

Biography & Abstract

Machine-learning guided low-N search for top variants for genome editor engineering

Dr. Athena Chu

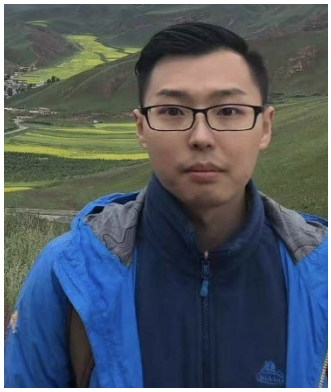
Prof. Alan Siu Lun Wong's Lab, The University of Hong Kong

Biography

Athena has joined Alan Wong's lab since 2020 as a postdoc. Her projects focus on protein engineering on various genome editor components. More specifically, she explores various methods to search or accelerate the search for beneficial substitutions for Cas9 protein optimization. She employed machine learning-guided screens with an active learning approach to effectively identify high-performance variants in a mutagenesis library. Such low-N learning method greatly reduces the experimental burden spent on cloning and screening variants with poor performance by up to 99%. Currently, she is exploring other machine learning models for protein design to create useful and diversified protein variants.

Abstract

We present a strategy to obtain the greatest number of best-performing protein variants with the least amount of experimental effort for mutagenesis screens to alleviate the experimental resources spent on cloning and testing non-functional variants. Our strategy uses zero-shot prediction and machine learning to guide multi-round sampling of top variants in the library. We found that four rounds of low-N pick-and-validate sampling with 12 variants for machine learning yielded the up to 92.6% accuracy in selecting the true top 1% variants in libraries with thousands of mutant combinations, while two rounds of 24 variants identify top variants with higher sequence diversity. Our strategy outperforms other state-of-the-art methods in terms of efficiency and accuracy and can be generalized for a range of protein-function inference including CRISPR genome editors.



Mutation-independent gene knock-in therapy targeting 5'UTR for autosomal dominant retinitis pigmentosa

Mr. Baoshan Liao

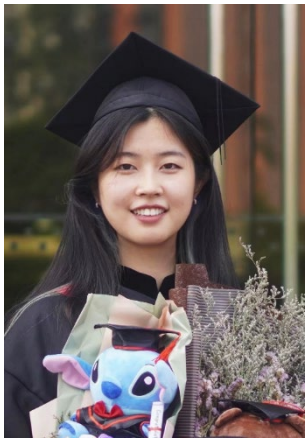
Prof. Wenjun Xiong's Lab, City University of Hong Kong

Biography

Mr Liao Baoshan holds a Master's degree from the University of Stirling, where he specialized in germ cell development in marine organisms using gene editing techniques. Currently, he is a PhD student in Professor Wenjun Xiong's lab, focusing on rescuing photoreceptors from degenerative retinal diseases through innovative approaches such as CRISPR/Cas technology and Müller glia reprogramming. Our ultimate goal is to advance therapies for vision-related disorders, with the hope that our research will transition from the lab to clinical applications in the future.

Abstract

Despite the recent success of gene supplementation therapy for monogenic recessive diseases, therapeutic approaches to treat dominantly inherited diseases fall behind. Here, we present a new gene knock-in (KI) therapy which exploits AAV-Cas9-mediated homology-independent targeted integration (HITI) of the wild-type coding sequence (CDS) into the 5' untranslated region (UTR), more specifically immediately upstream of the Kozak sequence, of the disease gene. We tested this approach in the heterozygous RhoP23H/wt mice, which carry the most common dominant point mutation found in the autosomal dominant Retinitis Pigmentosa (adRP) patients. We show that HITI-AAVs can mediate highly efficient gene insertion in mouse Rho 5'UTR in vivo. The RhoP23H/wt mice had significantly prolonged photoreceptor survival and visual function following the 5'UTR gene KI treatment. In summary, we developed a mutation-independent gene KI approach that targets 5'UTR of the disease gene and demonstrated its therapeutic potential to treat dominant diseases.



A Comparative Study of CRISPR-Based In vivo Gene Knock-In Strategies for Haemophilia B Treatment and Editing Outcomes in Genome

Miss Siqi Zhang

Prof. Bo Feng's Lab, The Chinese University of Hong Kong

Biography

Miss Zhang Siqi obtained her BSc in Biomedical Sciences from CUHK in 2021. She is current a PhD student at year 4, and will graduate in August 2025. Siqi research focuses on the CRISPR-Based In vivo Gene Knock-In for Haemophilia B Treatment, and thorough analysis of diverse targeting strategies and editing outcomes in genome.

Abstract

CRISPR-Cas9-mediated insertion of exogenous sequences into a targeted genomic locus showed promising potential to provide novel gene therapies to treat a wide range of inherited disorders. However, low integration rates and diverse editing outcomes remain the major hurdles for clinical translation. Our previous work has achieved effective knock-in of scAAV (self-complementary AAV)-delivered NHEJ (non-homologous end joining) donors at a CRISPR-induced DSB (double-stranded break). Stable plasma hFIX levels over 1000 ng/ml were detected for 48 weeks post injection. High-throughput RNA sequencing analysis unveiled ~3% reads containing hF9 insertion. In the meanwhile, significant byproducts were observed, including comparable levels of reverse insertion, frequent small indels at the cleavage sites, and rare AAV integration events.

GenomePAM directs PAM characterization and engineering of CRISPR-Cas nucleases

Mr. Miao Yu

Prof. Zongli Zheng's Lab, City University of Hong Kong

Biography

YU Miao is a Ph.D. candidate in the department of biomedical sciences at City University of Hong Kong. He earned his bachelor's and master's degrees in medicine from Sun Yat-sen University. His research focuses on genome editing, with a particular emphasis on CRISPR-related technologies. His work involves optimizing CRISPR/Cas and related sgRNA scaffold sequences to expand CRISPR targetability and enhance activity and exploring its applications in cancer treatment, with the goal of advancing clinical translation.

Abstract

The first step of DNA target site engagement for prokaryotic CRISPR-Cas nucleases is the recognition of protospacer adjacent motifs (PAMs). Characterizing the PAM requirements of different Cas enzymes is a bottleneck in the discovery of novel Cas proteins and their engineered variants in mammalian cell contexts. To overcome this challenge and to enable more scalable characterization of PAM preferences, here we developed a simple method named GenomePAM that allows for direct PAM characterization in mammalian cells. GenomePAM leverages repetitive sequences in the genome as target sites and thus does not require protein purification or synthetic oligos. Using a 20-nt protospacer that occurs ~16,930 times in every human diploid cell and is flanked by nearly random sequences, we demonstrate that GenomePAM can accurately characterize the PAM requirement of type II and type V nucleases, including the minimal PAM requirement of the near-PAMless SpRY and extended PAM for CjCas9. We further demonstrated the utility of GenomePAM to characterize the PAMs of novel Cas nucleases, type II TiCas9 and type V RuCas12a. Based on AlphaFold 3's prediction of TiCas9 PAM interaction domain structure, we engineered and relaxed its PAM sequence from the natural 'ACT' to 'ANT'. Beyond PAM characterization, GenomePAM allows for simultaneous comparison of activities and fidelities among different Cas nucleases on thousands of match and mismatch sites across the genome using a single gRNA; and provides insight into the genome-wide chromatin accessibility profiles in different cell types. The simplicity of GenomePAM should enable rapid and comprehensive characterization of the PAM requirements, potencies, and specificities of CRISPR-Cas enzymes.