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The fourth annual BRDS on genome editing and silencing for precision medicines

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Abstract

Precision medicine is promising for treating human diseases, as it focuses on tailoring drugs to a patient's genes, environment, and lifestyle. The need for personalized medicines has opened the doors for turning nucleic acids into therapeutics. Although gene therapy has the potential to treat and cure genetic and acquired diseases, it needs to overcome certain obstacles before creating the overall prescription drugs. Recent advancement in the life science has helped to understand the effective manipulation and delivery of genome-engineering tools better. The use of sequence-specific nucleases allows genetic changes in human cells to be easily made with higher efficiency and precision than before. Nanotechnology has made rapid advancement in the field of drug delivery, but the delivery of nucleic acids presents unique challenges. Also, designing efficient and short time-consuming genome-editing tools with negligible off-target effects are in high demand for precision medicine. In the fourth annual Biopharmaceutical Research and Development Symposium (BRDS) held at the University of Nebraska Medical Center (UNMC) on September 7–8, 2017, we covered different facets of developing tools for precision medicine for therapeutic and diagnosis of genetic disorders.

Keywords

Drug delivery; Genome-editing; Immunotherapy; Nanomedicines; Precision medicine

Introduction

Traditionally, medicines are developed on a “one size fits all” approach, which mostly fails to treat diseases, including diabetes, cystic fibrosis, and cancer [1]. Scientists have now begun to realize that there are often genetic variations among patients with the same disease. Unwinding genetic disparities responsible for various disease pathophysiological conditions and advent of tools to modulate gene expression fueled excitement for gene therapy. To target patients with different genetic variations, the scientist needs to develop tools to diagnose and to select the right patients for the right dose of therapeutics in a scientific way. As research has grown classier after the completion of human genome project, there is an exponential rise in nucleic acid-based therapeutics.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Precision medicine helps to understand disease complexity better to select therapies that work best for each patient. The need for personalized medicines has opened the doors for turning nucleic acids into therapeutics. There is an exponential rise in precision medicines, which has evolved as a promising approach to treat human diseases as it focuses on tailoring drugs to a patient's genes, environment, and lifestyle. The possibility of applying genome-editing methods to treat human diseases has attracted an enormous amount of scientific, clinical, and commercial interest. However, translating this potential into reality requires collaboration between scientists that are working on optimization of genome-editing techniques, gene therapy, and drug delivery approaches. Various strategies are used to modulate target gene expression including antagomirs or antimiRs, locked nucleic acids, siRNAs, miRNAs, miRNA sponges containing multiple tandem binding sites to target miRNA, Zinc-finger nucleases (ZFNs), transcription activator-like effector nucleases (TALENs), clustered regularly interspaced short palindromic repeats-associated protein (CRISPR-Cas), and small molecule inhibitors of miRNAs [2]. Recent advancement in life science has helped to better understand the effective manipulation and delivery of genome-engineering tools. The use of genome-editing system provides a way to create model cells, transgenic animals, engineer metabolic pathways, and treat human diseases that are difficult to tackle by conventional medications. Huge efforts have been devoted over the years to unravel the complex barriers in improving the efficiency of site-specific nucleases for clinical applications. Although gene therapy has the potential to treat or prevent both genetic and acquired diseases, it needs to overcome many obstacles before creating the overall prescription drugs. Among them, safe and efficient site-specific in vivo delivery of these personalized medicines remains a challenge due to their instability in the plasma, immunogenicity, poor cellular uptake, and intracellular trafficking, which necessitates the design of nano-delivery systems [3, 4]. These issues have diminished the impact of the “genomic revolution” declining the innovation of nucleic acid research and development.

Nanotechnology has made rapid advancement in the field of drug delivery, but the delivery of nucleic acids presents unique challenges. Also, designing efficient genome-editing tools with negligible off-target effects are in high demand for precision medicine. Although these efforts have not yet been fructified, the number of preclinical and clinical trials of gene medicines has grown in the recent years [5]. To further intensify the development of nucleic acid-based personalized medicines, an interdisciplinary approach is required.

With the aim of nurturing the relationships between biopharmaceutical industries, academic researchers, and students, the fourth annual Biopharmaceutical Research and Development Symposium (BRDS) was organized on September 7–8, 2017 at the University of Nebraska Medical Center (UNMC), Omaha. The theme of this year's symposium was “Genome Editing and Silencing for Precision Medicines” to explore different facets of developing tools for precision genome engineering in humans for therapeutic and diagnosis of genetic disorders. The symposium had interdisciplinary focus with the participation of scientists from diverse institutions across the world to share, discuss debate topics related to precision medicines, and potential approaches for its rapid clinical development. Dr. Ram I. Mahato, who introduced BRDS in 2014 [6], initiated the symposium by a welcome speech with a brief introduction about fourth annual BRDS. He is currently the Chair and Professor of the Department of Pharmaceutical Sciences at the UNMC.

At this symposium, 15 scientists were invited from across the world representing academia and industries for oral presentations. Also, young scientists for podium presentations and poster presenters were selected from various educational institutions, which helped them to promote the alliance of diverse interdisciplinary approach (Fig. 1). It also facilitated the knowledge sharing and intense discussion among the scientists with a broad interest in the field of gene therapy. Attendees presented their studies, novel technologies and approaches, clinical success, ethical issues related to genome editing, commercialization of scientific discoveries, and future directions.

The talk of the symposium was initiated by Dr. Vincent H.L. Lee from the Chinese University of Hong Kong. He introduced the progress made in the field of pharmaceutical sciences, drug discovery, and drug development. He mentioned that the use and development of the right drug, for the right patient, at the right time for the right period could help in better therapeutics. Per Dr. Lee, to transform drug discovery and drug development process, a paradigm shift in pharmacokinetics, pharmacogenomics, system biology, team-based healthcare practice, quality of life, high-throughput screening process, gene therapy, and tissue engineering to name some should be considered.

Genome-editing-based nanomedicines

The recently explored genome-editing approach, which uses the immune defense system of prokaryotes, has shown advantages over the previous regimes. The system is based on the CRISPR and CRISPR-associated *Cas* genes. This CRISPR-Cas-based genome editing is widely used across the living beings with several off-target effects. This system has revolutionized targeted genome editing and hold potential to give rise to an entirely new class of therapeutics. Due to its precise specificity, the system can be applied to target genomic-breakpoints at any desired location [7]. However, the off-target issues related to CRISPR-Cas system has been a concern, at least for using it as a therapeutic tool. Therefore, introducing a method to study the genome-wide off-target effects of CRISPR-Cas system is very promising. Among the invited speakers, Dr. Shengdar Tsai from St. Jude Children's Research Hospital initiated the talk on the genome editing. He introduces the development of cell-based and in vitro genome-scale methods to determine the low-frequency off-target activity of CRISPR-Cas system across genome called genome-wide unbiased identification of double-stranded breaks enabled by sequencing (GUIDE-seq) and circularization for in vitro cleavage reporting by sequencing (CIRCLE-seq) methods to determine the low-frequency off-target activity. GUIDE-seq is based on efficiently integrating short DNA tags into the sites of nuclease-induced DSBs followed by tag-specific amplification and high-throughput sequencing [8–10]. On the other hand, CIRCLE-seq is a method for selective sequencing of genomic DNA fragments cleaved by Cas9 in vitro [11]. Per Dr. Tsai, these methods provide an easy, rapid, and comprehensive way to identify genome-wide off-target mutations by CRISPR-Cas system.

Achieving therapeutic potential of CRISPR-Cas system requires its delivery to the target cells. Several vehicles have been used to successfully deliver ~ 4 kb Cas9 protein and its mRNA using adeno-associated virus (AAV) [12–14], lentiviral vectors [15], and non-viral vectors, such as cationic lipid-based vectors [16], cationic polymer-based vectors [17],

conjugated vectors [18], and the combination of viral and non-viral systems have also been studied. Continuing the session on genome editing, Dr. Daniel J. Siegwart from the University of Texas Southwestern Medical Center talked about the synthesis and development of zwitterionic amino lipids (ZALs) for safe and co-delivery of Cas9 mRNA and a single guide RNA (sgRNA). Using ZAL nanoparticles, Dr. Siegwart and his team were able to edit genomic DNA permanently with 95% decrease in protein level in vitro. In addition, when intravenously co-administered in genetically engineered mice containing a homozygous Rosa26 promoter Lox-Stop-Lox tdTomato (tdTO) cassette in all cells [19], Cas9 mRNA and sgRNA for LoxP (sgLoxP) were able to induce the expression of tdTO from the liver, kidneys, and lungs upon organs ex vivo imaging after 1 week [20]. Based on this observation, Dr. Siegwart concluded his talk by emphasizing the use of ZAL nanoparticles as a promising carrier of gene-editing systems.

Turning the path, Dr. Kamel Khalili from the Temple University Lewis Katz School of Medicine talked about CRISPS-Cas system for an HIV-1 cure. He shaded light on currently incurable acquired immunodeficiency syndrome (AIDS), the lack of therapy to eliminate the proviral DNA from the host genome, and the requirement of novel strategy. Since AIDS remains an incurable disease, he presented his work on the use of CRISPR-Cas system for deleting HIV-1 proviral DNA from the host genome and how it can protect cells against the spread of the virus and re-infection [21]. He concluded his talk with his intentions to utilize the strategy to cure AIDS in a clinical trial.

The genomic DNA is always under the influence of intrinsic and extrinsic agents, which leads to the generation of DNA double-strand break (DSB) [22]. DSB is one such damage, which recruits endogenous repair machinery for its repair by non-homologous end joining (NHEJ) or homology-directed repair (HDR). NHEJ leads to the introduction of insertion or deletion mutations (indels). The process is so active that no repair template or extensive DNA synthesis is needed for higher repair capacity in short time. On the other hand, HDR leads to the introduction of site-specific mutations or insertion of desired sequences through recombination of exogenously supplied donor DNA template to the target locus. Targeted genome editing via HDR is a robust approach in molecular biology [23]. However, the lower efficiency of engineered donor DNA template to correctly integrate into the desired target locus, lengthy selection process and adverse mutagenic effects hamper the use of HDR system. Genetic recombination in mammalian systems through HDR pathway is an inefficient process requiring cumbersome laboratory methods to identify the desired final events. Therefore, the development of an alternative tool, which slows or inhibit NHEJ can increase the repair efficacy through HDR. One such tool was the focus of the talk by Dr. Katherine Pawelczak from NERx Biosciences. She talked about the development of a series of small molecules, which can inhibit the DNA binding activity of a protein called Ku necessary for initiation of the NHEJ pathway. NHEJ depends on the conserved Ku proteins CKU-70 and CKU-80, which form heterodimers at the damaged site to protect the DNA. Once the Ku heterodimer is bound to the DSB ends, it serves as a scaffold to recruit the other NHEJ factors to the damage site and repairs DNA DSBs [24, 25]. Per Dr. Pawelczak, their molecules target the first step in NHEJ pathway and showed excellent potency in reducing the Ku-DNA binding activity. They also decreases DNA-PK activity (a catalytic activity that is dependent on Ku-DNA interactions) and NHEJ activity in numerous cellular

models. In addition, when used in combination with CRISPR-Cas, it allowed an increase in HDR-mediated gene insertion of large donor molecules, proving the potential of improving HDR-mediated DSB repair. Similarly, Dr. Rolf Turk from Integrated DNA Technologies (IDT) talked about the insertion of targeted repair template for the generation of knock-in strains or animal models, including insertion of reporter genes or specific mutations. As an evident, when the repair template consists of double-stranded DNA, the knock-in efficiency is usually very low [26]. Therefore, to overcome this low knock-in frequency, the use of single-stranded oligonucleotides could be an alternative as HDR templates. Although there is a complexity in synthesizing longer single-stranded oligonucleotides beyond 200 bases, the ability to use them as a repair template enables insertion of more massive sequences for HDR and targeted gene editing. Per Dr. Turk, IDT has developed a method to generate long single-stranded oligonucleotides templates up to 2 kb in length that is highly purified and sequence verified (IDT Megamers™ single-stranded gene fragments).

Many human disease-related genes have been mapped proving most diseases are genetically complex. Modeling the pathogenesis of patient's complex genetic alterations in specific cell types or organisms can ease the overall drug development process [27, 28]. The laboratory mouse is an advantageous model due to their small size and similar genomic-architect as that of a human. Mouse with specific gene modification is a valuable system to understand the function of a gene in diseases and discover improved methods to prevent, diagnose, and treat diseases. Until recently, manipulating mouse genes to study their function in health and illness was hard to achieve. However, the generation of specific disease models in animals that recapitulate the biological and clinical characteristics of humans by targeting specific locus in the genome has made CRISPR-Cas system an essential subject for studying various diseases [29]. Dr. Channabasavaiah Gurumurthy from UNMC, introduced *Easi*-CRISPR (*Efficient additions with ssDNA inserts*-CRISPR), a strategy of using long single-stranded DNA as a donor template to solve the major issues related to animal genome engineering by the inefficiency of targeted DNA cassette insertion [26]. Per Dr. Gurumurthy, this method can bypass all the significant steps of animal transgenesis.

No discussion on genome editing is complete without thinking about its ethical issues. Therefore, to fill this gap, we invited Carol Szczepaniak, a president of Nebraska coalition for ethical research (NCER), to point out the impact CRISPR-Cas technology on multiple disciplines from agriculture to medicine as a boon or bane. Per Szczepaniak, the excitement of being able to treat incurable diseases overshadows the consequences of permanent alteration of the human genome, eugenics applications, non-therapeutic designer babies, and militarized transhumanism. She pointed toward CRISPR-Cas gene-editing studies in China, which led to uncontrolled and unintended editing. Though the prospects are unlimited, the safety and ethical concerns must be addressed and monitored within the scientific community. She ended her talk saying that the scientific community must walk a fine line, making ethical decisions that improve humanity without jeopardizing it.

Other therapeutics and drug delivery approaches

In addition to genome editing and silencing for precision medicines, the fourth annual BRDS was also framed to cover other critical therapeutic approaches including immunotherapy and design of the delivery systems.

Immunotherapy

Understanding the role of immune system in cancer has grown tremendously over the past few decades. Genetically engineered immune cells have been studied as a treatment option for multiple diseases including HIV and cancer. Invariant natural killer T (iNKT) cells are a small population of $\alpha\beta$ T lymphocytes, which are derived from hematopoietic stem cells (HSCs) and develop in the thymus. iNKT cells have shown potential to play important roles in regulating diseases like cancer, allergies, and many others [30]. When stimulated, these cells release a lot of cytokines such as IFN- γ and IL-4. These cytokines then activate various immune effector cells like natural killer (NK) cells and dendritic cells of the innate immune system, as well as a CD4 helper and CD8 cytotoxic conventional $\alpha\beta$ T cells of the adaptive immune system via activated dendritic cells. This unique mechanism of iNKT cells enables them to attack multiple diseases independent of antigen and MHC restrictions, making them attractive universal therapeutic agents [30, 31]. However, their low population in human restricts their use as therapeutics. To overcome this challenge, Dr. Lili Yang from the University of California Los Angeles came up with a method of generating a large quantity of iNKT cells in mice through T cell receptor gene engineering of HSCs in a short time. Following a two-step developmental pathway, first in thymus and then in the periphery, Dr. Yang and her team were able to generate a clonal population of iNKT cells with typical iNKT cell phenotype and functionality. Using these clonal cells, they were able to protect mice from tumor metastasis. Per Dr. Yang, her method has opened a door for a larger supply of engineered iNKT cells and iNKT cell-based immunotherapy [32].

Continuing a talk on immunotherapy, Dr. Yoshinobu Takakura from Kyoto University, Japan shed a light on exosomes as a potential delivery vehicle of drugs for tumor antigen-based immunotherapy. Exosomes are extracellular vesicles, which function as a mode of intercellular communication and transfer proteins, lipids, and variety of nucleic acids between the cells. The cancer cells derived exosomes as cancer vaccine contains endogenous tumor antigens, which induces the antitumor immunity by transferring tumor antigens to antigen-presenting cells (APCs) [33, 34]. Therefore, the efficient and simultaneous delivery of tumor cell-derived exosomes and adjuvant to the same APCs could effectively induce potent tumor antigen-specific immune responses. Based on this assumption, Dr. Takakura and his team modified B16BL6 cells to obtain iodine-125 (^{125}I) based on streptavidin (SAV) —biotin-labeled exosomes to study the pharmacokinetic profiles of exogenously administered exosomes, an important issue for the development of exosome-based delivery systems [35]. They also developed an exosome-based antigen-adjuvant co-delivery system using genetically engineered tumor cell-derived exosomes containing endogenous tumor antigens and CpG DNA, a potent adjuvant. Per Dr. Takakura, vaccination with CpG DNA-modified exosomes exhibited strong antitumor immunity, which can be used as a powerful approach for cancer immunotherapy [36].

Dr. Andrew Godwin from the University of Kansas talked about the role of the exosomes in cancer initiation, progression, and drug resistance. Per Dr. Godwin, he and his team fabricated the first microfluidic platform called lab-on-a-chip that integrates specific immunocapture and targeted protein analysis of circulating exosomes [37]. He further discussed the ways to exploit these exosomes and their payloads of proteins and nucleic acids for cancer diagnostics using miniaturized biomedical assays.

Design of the drug delivery vehicles

Shifting gears, the next topic for discussion was the design of drug delivery approaches. Dr. Jianzhong Du from Tongji University, Shanghai, China initiated the discussion by talking about the use and development of a nuclear envelope-like polymer capable of loading and delivering proteins, RNA, and DNA [38]. He further shed light on the design of noncytotoxic asymmetrical cancer-targeting polymer vesicles based on R-poly(L-glutamic acid)-block-poly(caprolactone). This polymeric system, which contains cancer-targeting outer corona and a Gd(III)-chelating and drug-loading-enhancing inner corona, exhibited an extremely high T_1 relaxivity (42.39 mM⁻¹s⁻¹, eight-fold better than DTPA-Gd). With 52.6% loading of doxorubicin, this vesicle exhibited twofold better antitumor activity than free doxorubicin. Since most tumors are heterogeneous in nature with a small population of CSCs providing enhanced EMT, tumor initiation, and chemoresistance properties [39]. Dr. Du and his team designed an EpCAM (epithelial cell adhesion molecule)-antibody-labeled noncytotoxic polymer vesicle, which showed high delivery efficacy of doxorubicin and siRNA to overcome drug resistance by silencing the expression of oncogenes specific to the CSCs [40]. Per Dr. Du, compared to non-CSCs-targeting vesicle, the doxorubicin or siRNA loaded CSCs-targeting vesicle exhibited excellent CSCs killing and tumor growth inhibition.

Continuing the talk on delivery vehicles, Dr. Won Jong Kim from Pohang University of Science and Technology, Republic of Korea gave a lively talk on intelligent nanomedicine for the delivery of therapeutics with reduced side effects and enhanced efficacy. Per Dr. Kim, they have explored various stimuli-responsive nanomedicines, including inorganic nanoparticles and biopolymers, for the delivery of a wide range of therapeutic agents such as chemotherapeutics, genes, and the gas molecules. In another study, they have used polydopamine and light-responsive nitric oxide (NO)-releasing nanoparticles where the light-responsive gatekeeper is composed of a pH-jump reagent as an intermediary of stimulus and a calcium phosphate (CaP) coating as a shielding layer for NO release. The acid generated by light irradiation triggers uncapping of the gatekeeper and release of NO and accelerates the degradation of the CaP-coating layers on the nanoparticles [41]. At the end, Dr. Kim emphasized that this approach could provide the spatiotemporal controlled NO delivery.

The talk of the second day was ended by Dr. Michael Dixon from UNeMed Corporation, a company that works with faculty, students, and staff of UNMC to help commercialize innovative and new ideas that have the potential to improve public health. He reminded the attendee that there are myriad of critical decisions and pathways to traverse before getting any research out to the public as a product. He further discussed the critical role of

intellectual property and how educational institutions and companies work together to achieve the monumental task of developing a discovery into a lifesaving drug.

Podium presentations by emerging scientists

Besides oral presentations by world leaders, four young scientists were selected for podium presentations at this year's annual BRDS. Graduate research assistants from the South Dakota State University and the University of Nebraska-Lincoln shared their research on the development of site-directed polymer-drug complexes for modulating gut innate immune system to prevent and treat inflammatory bowel diseases and engineering lipid-based nanoparticles for targeting inflammation site in atherosclerosis, respectively. Following these, a research instructor and a postdoctoral research associate from UNMC talked on colloidal stability and spectral properties of carbazole-based fluorescent nanoaggregates and design, synthesis and biological evaluation of potent analogs of GDC-0449 for the treatment of pancreatic cancer, respectively [42]. In addition to these presentations, more than 17 posters were enthusiastically presented by scientists and students from and outside of UNMC.

Finally, the symposium was concluded by Drs. Courtney Fletcher, Dean, College of Pharmacy and Tatiana K. Bronich, Co-director of the Center for Drug Discovery and Nanomedicine (CDDN), UNMC. They summarized the accomplishments of this noteworthy symposium and acknowledged their colleagues, scientists, and students for their active participation. Moreover, they shared their experiences, achievements, and the benefits of international collaboration in research. Additionally, awards were handed to the best podium and poster presenters to encourage research activities among young scientists.

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Fig. 1.
Participants of the fourth annual BRDS