Giridhara R. Jayandharan *Editor*

Gene and Cell Therapy: Biology and Applications

Springer

Gene and Cell Therapy: Biology and Applications

Giridhara R. Jayandharan
Editor

Gene and Cell Therapy: Biology and Applications

Springer

Editor
Giridhara R. Jayandharan
Department of BSBE
IIT-Kanpur
Kanpur, Uttar Pradesh, India

ISBN 978-981-13-0480-4 ISBN 978-981-13-0481-1 (eBook)
https://doi.org/10.1007/978-981-13-0481-1

Library of Congress Control Number: 2018950550

This Springer imprint is published by the registered company Springer Nature Singapore Pte Ltd.
The registered company address is: 152 Beach Road, #21-01/04 Gateway East, Singapore 189721, Singapore

Preface

Recent advances in stem cell biology, nanotechnology, and gene therapy have created new avenues for therapeutics. The availability of molecular therapeutics that rely on delivery of DNA, RNA, or proteins, harnessing enhanced delivery with nanoparticles, and the regenerative potential of stem cells (adult, embryonic, or induced pluripotent stem cells) have made a tremendous impact on translational medicine. The attached book chapters cover the various strategies for molecular and cellular therapies for human disease, including their advantages and challenges to their widespread applications. Potential solutions to these issues have been discussed elaborately. This book will provide an overview of advances on novel therapeutics and provide specific examples of disease conditions where these strategies have been translated to the clinic. The chapters also highlight state of the art into current research aspects of molecular and cell therapies.

A summary of the chapters covered in this book is given below.

Part I: Gene Therapy

Chapter 1. Retroviral Vectors in Gene Therapy

Chitra Gopinath · Trupti Job Nathar · Everette Jacob Remington Nelson
Gene Therapy Laboratory, SMV124A, Department of Integrative Biology, School of Biosciences and Technology, Vellore Institute of Technology, Vellore, TN, India

Viral vectors are the most effective means to deliver genes into cells. Evolution of viruses over the years has enabled them to adopt several strategies not only to enter but also infect a wide range of cells. Making use of this property, scientists have manipulated viruses to express therapeutic genes. These viruses serve as vehicles for gene delivery and are referred to as viral vectors. The ones that are currently used in gene therapy include retroviral vectors, lentiviral vectors, adenoviral vectors, adeno-associated viral vectors, and foamy viral vectors. Each of them has their own salient features that are both beneficial and harmful. Retroviral vectors belonging to the family *Retroviridae* were among the first viral vectors used in gene therapy clinical trials. Their genetic material is in the form of RNA. The ability of

retroviruses to integrate into the host genome makes them a permanent resident of the cell. The *Retroviridae* family is further divided into two subfamilies and seven genera, out of which gammaretrovirus, lentivirus, and foamy virus are the most widely used. Gammaretroviral vectors which have been part of nearly 21% of gene therapy clinical trials were developed from the prototype Moloney murine leukemia virus and hence referred to as MoMLV-based retroviral vectors. The gammaretroviral vector genome ranges in size from 9 to 11 kb and is composed of two long terminal repeats (LTRs) one each at the 3′ and 5′ ends and three essential genes, namely, *gag*, *pol*, and *env*, which code for proteins required for the viral packaging. Reports of insertional mutagenesis and clonal proliferation due to the integration of gammaretrovirus into the LMO2 proto-oncogene raised concerns about the safety of its application for human gene therapy. Another drawback of this virus is that it can transduce only rapidly proliferating cells. So, in order to successfully target terminally differentiated or largely quiescent cells such as stem cells, lentiviruses are used as they could infect both dividing and nondividing cells the fairly efficiently. Unlike gammaretroviruses, lentiviruses do not require cells to be in the active mitosis while entering. Over the past three decades, three different generations of lentiviral vectors have been developed, each generation significantly improved over the preceding one. Safety has always been a concern with the use of all viral vectors due to the adverse events reported such as immune response in the case of adenoviral vectors and insertional mutagenesis in the case of retroviral vectors. Recently, third-generation self-inactivating (SIN) lentiviral vectors have been proven to be very efficient and also safer when compared to gammaretroviral vectors which were used in earlier clinical trials. Despite integrating into active transcription units, lentiviral vectors were reported to have a safer integration profile compared to gammaretroviral vectors. Foamy viruses belong to the genera of *Spumavirus*. Analysis of integration sites of foamy viruses in HSCs has shown a unique and safe integration profile compared to both gamma and lentiviruses. Reports from ongoing clinical trials might answer emerging questions related to their safety and efficacy in human gene therapy applications.

Chapter 2. Adeno-associated Virus Vectors in Gene Therapy

Bertin Mary · Nusrat Khan · Sathyathithan Arumugam · Himanshi Saxena
Mohit Kumar · Paramasivam Manimaran · Sourav Chattopadhyay
G. R. Jayandharan
Department of Biological Sciences and Bioengineering,
IIT-Kanpur, Kanpur, Uttar Pradesh, India
e-mail: jayrao@iitk.ac.in

Adeno-associated virus (AAV) belongs to family Parvoviridae and the genus Dependovirus. To date 13 different human serotypes (AAV1 to AAV13) and more than 100 serotypes from nonhuman primates have been discovered. The

nonpathogenic nature of this virus, the persistence of viral infection in dividing and nondividing cells, and the availability of multiple serotypes have enhanced the utility of AAV as a delivery vehicle for gene therapy applications. However, the recent success in phase I/II clinical trials has also highlighted the issues related to the host- and vector-related immune responses that preclude the universal application of this promising vector system. A fundamental insight into the mechanism by which AAV infects the host cell and a thorough understanding of the immediate and long-lived cellular responses to AAV infection will offer clues and help design better intervention strategies to improve the therapeutic efficiency of AAV vectors. This chapter will review the biology of AAV-host cellular interactions and outline their application in the development of improved AAV vector systems for human gene therapy.

Chapter 3. Nanointerventions for Gene Therapy

K. Uma Maheswari
Centre for Nanotechnology & Advanced Biomaterials (CeNTAB),
School of Chemical & Biotechnology,
SASTRA University,
Thanjavur, Tamil Nadu, India
e-mail: umakrishnan@sastra.edu

Vadim Annenkov
Limnological Institute, Siberian Branch of the Russian Academy of Sciences,
Irkutsk, Russia

Translation of gene therapy from bench to bed has become a reality due to nanoparticle-mediated delivery of the therapeutic genes. The therapeutic potential of naked oligonucleotides has been limited by their poor stability as well as off-targeting effects. However, complexation of the anionic oligonucleotides in cationic nanodimensional carriers has enhanced their stability. Moreover, modification of the nanocarrier surface with targeting ligands has reduced off-targeting effects and enhanced site-specific delivery. The carriers can also be programmed to release their cargo over an extended period of time. In addition, the choice of the carrier material or selective incorporation of molecules that can facilitate endosomal escape ensures higher transfection efficiency for the carriers. Though non-viral carriers have been in general considered less efficient than viral carriers, an emerging paradigm in the field of nanotechnology has introduced hybrid carriers. Here, a viral vector is encapsulated in a non-viral nano-dimensional carrier thereby retaining the high transfection efficiency of viral-based delivery systems and also poses a lower risk to the biological system. This chapter elaborates on the various types of nanocarriers and surface modifications that have been attempted for efficient delivery of the therapeutic gene to the target. Shortcomings of the existing systems and future perspectives for nanoparticle-mediated gene delivery have been discussed. A separate section on clinical trials involving nanoparticles for gene delivery has also been included.

Chapter 4. Viral- and Non-viral-Based Hybrid Vectors for Gene Therapy

Manohar Mahato · Praveen Kumar Vemula
Institute for Stem Cell Biology and Regenerative Medicine (inStem),
Bangalore, India
e-mail: praveenv@instem.res.in

Giridhara R. Jayandharan
Department of Biological Sciences and Bioengineering,
IIT-Kanpur,
Kanpur, UP, India

Gene therapy offers a great potential for the treatment of genetic diseases as well as acquired diseases by means of delivering therapeutic nucleic acids inside the cell. To deliver nucleic acids, broadly two strategies have been employed by using viral vectors and non-viral vectors. The viral vectors exhibited high transduction efficacy both in vitro and in vivo. The non-viral vectors composed of mainly cationic polymers and lipids which provide efficient condensing capability against negatively charged nucleic acids and low cytotoxicity. Till date, >2300 clinical trials for gene therapy are going on worldwide, approximately 70% using viral vectors and remaining with non-viral vectors. The immunogenicity and non-targeting abilities are the biggest hurdles in terms of safety and efficiency for successful therapy with these vectors. These two classes of vectors have their own advantages as well as disadvantages which hinder their therapeutic endpoint in clinical trials. Now, researchers have made attempts to form virus encapsulated in chemical vectors which are called as hybrid vectors. These hybrid vectors have immense potential to evade host immune system by masking the immunogenic epitopes present on viral vectors. The molecules or scaffold which is used for encapsulating virus enhance their targeting ability and sustained release to the targeted tissue. The hybrid vectors, a combination of viral and chemical vectors, form a new class of gene delivery vectors which overcome the limitations of each vector and simultaneously augment desirable features such as targeting ability, low immunogenicity, cytotoxicity, higher payload, and ability to deliver more than one transgene. The hybrid vectors should retain characteristics of each vector in order to achieve optimal tissue targeting and gene delivery with minimal toxicity. To achieve therapeutic endpoint with the hybrid vectors, development of such hybrid vectors requires extensive understanding of physicochemical properties after coating virus with chemical analogues and their optimal ratio as well. These aspects will be discussed in this chapter.

Chapter 5. Pharmaco-Gene Therapy

Martin H. M. Sailer
Department of Biomedicine, Pharmacenter,
University of Basel, Basel, Switzerland

Ganesh Ram Sahu
GROW Research Laboratory, Narayana Nethralaya Foundation,
Bangalore, India

Vellore Institute of Technology, Vellore, India

Arkasubhra Ghosh
GROW Research Laboratory, Narayana Nethralaya Foundation,
Bangalore, India
e-mail: arkasubhra@narayananethralaya.com

There are numerous disease conditions emerging from mutations in genes or alterations in gene expression for which gene-based therapies provide the most promising treatment modality. However, expression of therapeutic genes and proper localization of the expressed proteins require a complex interactive environment within the target cells or tissues for ultimately being clinically applicable. Our knowledge of molecular pathways, cell biology, and the immune system has helped in the development of a variety of drugs that can modulate these processes. Historically, gene therapies and pharmacologic therapies have remained isolated methods of tackling a disease. In this chapter, we will provide an alternate perspective that converges both schools of thought. In specific cases, if conducive to the disease mechanism and the underlying pathology, a pharmacologic drug can help enhance and regulate a gene therapy or vice versa. Such combined approaches can hence increase the specificity and efficiency of the final therapy, which may be much better than the individual ones. There are many hurdles to such dual-modality processes and hence must be employed after careful evaluation of the proposed positive and negative outcomes at a molecular level. We will provide specific examples of such collaborative pharmacologic and gene-based therapeutic strategies and discuss how similar strategies may be beneficial in different diseases.

Chapter 6. Aptamer as Therapeutics for Cancer with Focus on Retinoblastoma

Nithya Subramanian
PNAC Department,
MRC – Laboratory of Molecular Biology, Cambridge, UK

Akilandeswari Balachandran
Department of Nanobiotechnology,
Vision Research Foundation, Chennai, India

Krishnakumar Subramanian
L&T Department of Ocular Pathology, Department of Nanobiotechnology,
Vision Research Foundation, Chennai, India
e-mail: drkk@snmail.org

Aptamers are composed of oligonucleotide moiety either deoxyribonucleotides (DNA aptamer) or ribonucleotides (RNA aptamer). This single-stranded oligonucleotide molecule folds in specific three-dimensional conformations that enables them to bind specifically to target molecules. These aptamers are highly specific which enables them to differentiate between different conformations of the same protein. Aptamers have several advantages such as smaller size, less immunogenicity, less toxicity, increased stability upon modifications, less production cost compared to antibodies, long shelf life, and highly specific towards the target. The selections of these aptamers are done mainly against cell surface receptors. These aptamers are selected in vitro against targets based on SELEX (systematic evolution of ligands by exponential enrichment) approach. They can also be chemically modified to reduce their size, increase specificity, and also conjugate other molecules. These aptamers can also be modified with fluorescent molecules at 3′ and 5′ positions enabling their use in imaging purposes. The first aptamer approved by FDA is pegaptanib (Macugen) which is used for treating age-related macular degeneration. For cancer therapy, many aptamers are in clinical trials which include anti-PSMA aptamer (A10), anti-nucleolin aptamer (AS1411), anti-mucin aptamer (MUC-1), and anti-stromal cell-derived factor-1 aptamer (NOX-A12). Other than cancer therapeutics, aptamers find major applications in the field of biosensors and bioimaging as well.

Part II: Cell Therapy – "With Emphasis on Gene-Modified Cell Therapies"

Chapter 7. SETting up Methylation in Mammalian Cells: Role of Histone Methyltransferases in Disease and Development

Abhishek Mohanty · Shravanti Rampalli
Centre for Inflammation and Tissue Homeostasis,
Institute for Stem Cell Biology and Regenerative Medicine (inStem),
Bangalore, India
e-mail: shravantird@instem.res.in

It is now increasingly evident that the alterations in epigenetic factors have a major impact on human development. Therefore, a substantial amount of efforts are invested at academia and industry toward understanding the mechanism and treatment of such diseases. Embryonic stem cells and, their counterparts, induced pluripotent cells have revolutionized the field of regenerative medicine. Specifically, iPSC technology provides us with the unique tools that are needed for personalized medicine. This chapter will highlight the role of epigenetic regulators in the induction of pluripotency and how their perturbations impact development and disease.

Chapter 8. Hope or Hype: Stem Cells as Therapeutics in Retinal Degenerative Diseases

Parameswaran Sowmya
Radheshyam Kanoi Stem Cell Laboratory, KNBIRVO,
Vision Research Foundation, Sankara Nethralaya,
Chennai, India
e-mail: drpsowmya@snmail.org

"Netram Pradhanam Servendriyanam"; "Eyes are the precious gift given to humans by the Almighty" – verse from Bhagavad Gita emphasizes the importance of vision for the mankind. The most recent statistics from the World Health Organization (WHO) reveals that 39 million are blind worldwide, signifying the problem and a need to initiate formidable approaches to address the issue. Almost all the ocular diseases involving the retina, the innermost layer of the eye composed of light-sensitive tissue, is characterized by degeneration of retinal cells. The treatment for retinal degenerative diseases is impeded for the want of suitable cells to replace those that are getting degenerated in response to the disease. Stem cell therapy offers a unique opportunity to replace the damaged cells with new ones. Stem cell-based therapeutic approaches can be broadly classified as exogenous and endogenous. The former utilizes exogenous stem cells, such as mesenchymal stem cells, neural progenitors, embryonic stem cells, and induced pluripotent stem cell-derived retinal progenitors that are transplanted into the degenerating retina. The latter approach utilizes activation of endogenous stem cells present in the retina for replacing the degenerating cells. In this book chapter, the key concepts involving both the exogenous and endogenous stem cells for retinal degenerative diseases and their potential advantages and the limitations will be discussed. The outcome of the recent clinical trials along with the future directions and the challenges of stem cell-based therapies will be briefly covered.

Chapter 9. Haploidentical Stem Cell Transplantation

Narendra Agrawal · Dinesh Bhurani
Department of Hematology, Rajiv Gandhi Cancer Institute &
Research Centre,
Delhi, India

The use of hematopoietic stem cells for transplantation has become standard treatment modality for a wide variety of acquired and congenital disorders of the hematopoietic system. HSCT not only replaces defective/diseased hematopoietic tissue but also provide antitumor immunity in malignant conditions.

Over the span of five decades, there have been many developments in the field of HSCT like high-resolution HLA typing, unrelated donor pools, cord blood banks, preferable use of peripheral blood stem cells and marrow stem cells for different sets of diseases, tailored conditioning regimes to suit different diseases and different ages, better preventive measures for graft versus host disease (GvHD) and opportunistic infections, and managing transplant across HLA disparate pairs. We will discuss the promise and implications of this form of therapy for various hematological disorders.

Contents

About the Editor

Dr. Giridhara R. Jayandharan is currently working as an Associate Professor at Department of Biological Sciences and Bioengineering, Indian Institute of Technology, Kanpur, India. Dr. Jayandharan completed his Ph.D. from Christian Medical College, Vellore, India, in the year 2006 and subsequently worked as post-doctoral fellow at University of Florida, Gainesville, USA, between the year 2007 and 2009 where he worked extensively on gene therapy. Before joining the coveted Indian Institute of Technology, he worked as a faculty member in the Department of Hematology and also at Center for Stem Cell Research, Christian Medical College, Vellore, India. His main area of research includes gene transfer and therapy, human molecular genetics, and virology.

Dr. Jayandharan is a prolific publisher as he has over 60 papers in peer-reviewed high quality journals. He is recipient of several awards and honors. Most notable awards received by him are Wellcome Trust DBT senior fellowship, Swarnajayanthi fellowship from Department of Science and Technology, Government of India, Young scientist award, YIM Boston, USA; Senior Innovative Young Biotechnologist award, Department of Biotechnology, Government of India; Bayer Hemophilia Early Career Investigator Award, Bayer Inc, USA etc.

Dr. Jayandharan is a member of several notable academies/societies such as International Society for Thrombosis and Hemostasis, European Society for Gene and Cell Therapy, American Society for Gene and Cellular Therapy.

Part I

Gene Therapy