Preface

The mechanic that would perfect his work must first sharpen his tools.
Confucius (c. 551 BC–479 BC), a Chinese philosopher

Give us the tools, and we will finish the job.
Winston Churchill (1874–1965)

Gene therapy offers many conceptual advantages to treat muscle diseases, especially various forms of muscular dystrophies. Many of these diseases are caused by a single gene mutation. While the traditional approaches may ameliorate some symptoms, the ultimate cure will depend on molecular correction of the genetic defect. The clinical feasibility of gene therapy has been recently demonstrated in treatment of a type of inherited blindness. By delivering a therapeutic gene to the retina, investigators were able to partially recover the vision in a disease once thought incurable. Compared to retinal gene therapy, muscle gene therapy faces a number of unique challenges. Muscle is one of the most abundant tissues in the body. An effective therapy will require systemic infusion and targeted muscle delivery of huge amounts of therapeutic vectors. Severe inflammation associated with muscle degeneration and necrosis may further complicate immune reactions to the viral vectors and the therapeutic gene products. Furthermore, the vast majority of our current knowledge on muscle gene therapy is obtained from rodent models. Although these proof-of-concept studies have provided the critical foundation, the results are not easily translatable to human patients. With this in mind, we compiled this collection of muscle gene therapy methods and protocols with the intention of bridging the translational gap in muscle gene therapy.

The book is divided into three sections. The first section includes basic protocols for optimizing the muscle gene expression cassette and for evaluating the therapeutic outcomes. The chapters on the muscle-specific promoters and codon optimization outline strategies to generate powerful cassettes for muscle expression. Four chapters are devoted to end-point analysis. These include the use of epitope-specific antibodies, noninvasive monitoring of myofiber survival, and physiology assays of skeletal muscle and heart function.

Technology breakthroughs are the driving force in muscle gene therapy. Early muscle gene transfer studies were largely performed using vectors based on retrovirus, adenovirus, or plasmid DNA. Inherent limitations of these vectors (such as low transduction efficiency, transient expression, and a strong immune response) suggest that they are unlikely to meet the clinical need. These traditional gene delivery vehicles have now been replaced with the robust adeno-associated viral vector (AAV), oligonucleotide-mediated exon-skipping, and novel RNA-based strategies such as microRNA and RNA interference. The second section of this book is dedicated to the new developments in muscle gene therapy technology. Two chapters describe new strategies to generate muscle-specific AAV vectors by *in vivo* evolution and capsid reengineering. Two chapters provide methods for optimizing exon-skipping, and three chapters detail different applications of RNA-based approaches in muscle gene therapy.

Considering the importance of large animal studies, it is not surprising that the bulk of the protocols are devoted to muscle gene transfer in large animals models. In the last section, ten chapters provide step-by-step guidance on muscle gene delivery in swine, ovine, canine, and nonhuman primates. Methods include local delivery, isolated limb perfusion, myocardial gene transfer, and whole body systemic delivery. Ages range from fetal and neonatal to adult subjects.

In summary, this book presents a comprehensive collection of state-of-the-art muscle gene therapy protocols from leaders in the field. I would also like to mention that this collection of muscle gene therapy techniques complements the recently published book entitled "Muscle Gene Therapy" (Duan D eds., Springer, 2010, ISBN 978-1-4419-1205-3). Together, they will serve as a valuable resources for graduate students, postdoctoral fellows, and principle investigators who are interested in muscle gene therapy.

I would like to thank the contributors of each chapter for their excellent contributions. There is no doubt that these hard-to-find techniques, tricks, and the hands-on experience from the leading investigators will play an important role in bench-side to bedside translation of muscle gene therapy. I would like to thank Dr. John Walker, the series editor, for his guidance in the development of this book. I would like to thank Ms. Karen Ehlert for her administrative assistance in the final stage of preparation.

I am also very grateful to the National Institutes of Health and the Muscular Dystrophy Association for the funding of muscle gene therapy studies in my laboratory. I also thank the Parent Project Muscular Dystrophy and Jesse's Journey, The Foundation for Gene and Cell Therapy for their recent support in expanding our research in developing muscular dystrophy gene therapy. I am also much indebted to the patients and their families. I truly believe their dream will one day come true.

Finally, I'd like to dedicate this book to boys like Mark McDonald, they are our driving force.

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