

METHODS IN MOLECULAR BIOLOGY

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Gene Therapy for Neurological Disorders

Methods and Protocols

Edited by

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Preface

Gene therapy of the nervous system, a technique once utilized by a few select laboratories, is now a commonplace research tool used around the world. Not only is gene therapy a useful utility in treating and creating preclinical models, but this technology has also demonstrated success in the clinic, in terms of both safety and efficacy [1, 2].

Gene therapy is a valuable tool that is being increasingly utilized to model neurodegenerative disorders [3]. One reason for this is the inherent ability of gene therapy to control genetic expression in both a spatial and temporal manner. For example, using this precision of gene therapy to model neurodegenerative disorders enables researchers to overcome any developmental compensations that may occur with germ line manipulations [4, 5], to create lesions that are restricted to one hemisphere or specific circuits, and to easily titrate the genetic material of interest [6], among other benefits. Of course, these benefits of gene therapy also translate to the use of gene therapy for the delivery of therapeutic genes in preclinical models of neurological disorders [7–11]. That being said, after over 15 years of experience in gene therapy, it has become clear to me that a significant amount of crucial knowledge necessary to design and execute a *successful* gene therapy experiment often fails to be disseminated in a normal format (i.e., via scientific manuscripts). Rather, this esoteric, yet essential knowledge is either briefly mentioned or solely propagated via word of mouth. Therefore, it is all too common that studies involving gene therapy manipulations produce results that vary between investigators (e.g., Ref. 12). Although such discrepancies are not the result of any wrongdoing, their occurrence adds to the “mysticism” sometimes associated with gene therapy and could serve to reduce the enthusiasm for taking on similar projects in the future. Thus, one purpose of this book is to dispel any confusion and provide a clear and detailed road map of how to successfully design and execute a gene therapy experiment in order to obtain consistent results.

As science progresses and new discoveries are made, the boundaries of gene therapy are rapidly expanding: Gene therapy vehicles are continuously undergoing development and are becoming more readily available, delivery methods are continuously being developed, and transgene cassettes are becoming more and more refined. This leaves the researcher with a plethora of decisions that must be considered before undertaking a gene therapy experiment. In this volume I have invited experts from around the world to share their expertise in finite areas of neurological gene therapy. The compilation of protocols and instructive chapters in this book are intended to give researchers, clinicians, and students of all levels a foundation upon which future gene therapy experiments can be designed. When one designs experiments involving gene therapy of the nervous system, several aspects need to be considered before experiments are designed: What delivery vehicle do you use? Will you produce this vector? How will you ensure that your vector retains stability? What expression system best fits your needs? What route will you choose to deliver your gene therapy agent? How will you model the neurodegenerative disorder that you aim to investigate, and what are the proven methods to treat these disorders in preclinical models? This book is aimed to address all these important considerations as well as to disseminate the

aforementioned bits of arcane information that are very important to consider during the course of experimentation.

Finally, the penultimate goal for many gene therapists is to see their product eventually end up in the clinic as a treatment for neurological disorders. Although gene therapy has progressed to the clinic, this is not a straightforward path as several variables such as age and disease status have to be considered. Several chapters in this volume will also discuss special considerations that need to be addressed when translating experimental approaches to the clinic.

1. Maguire AM et al. (2009) Age-dependent effects of RPE65 gene therapy for Leber's congenital amaurosis: a phase 1 dose-escalation trial. *Lancet* 374: 1597–1605
2. Marks WJ Jr. et al. (2010) Gene delivery of AAV2-neurturin for Parkinson's disease: a double-blind, randomised, controlled trial. *Lancet*. 9: 1164–1172
3. Kirik D et al. (2002) Parkinson-like neurodegeneration induced by targeted overexpression of alpha-synuclein in the nigrostriatal system. *J Neurosci* 22: 2780–2791
4. Gorbatyuk OS et al. (2010) In vivo RNAi-mediated alpha-synuclein silencing induces nigrostriatal degeneration. *Mol Ther* 18: 1450–1457
5. Kanaan NM, Manfredsson FP (2012) Loss of functional alpha-synuclein: a toxic event in Parkinson's disease? *J Parkinsons Dis* 2:249–267
6. Manfredsson FP et al. (2009) Tight Long-term dynamic doxycycline responsive nigrostriatal GDNF using a single rAAV vector. *Mol Ther* 17: 1857–1867
7. McBride JL et al. (2003) Structural and functional neuroprotection in a rat model of Huntington's disease by viral gene transfer of GDNF. *Exp Neurol* 181: 213–223
8. Gombash SE et al. (2012) Striatal pleiotrophin overexpression provides functional and morphological neuroprotection in the 6-hydroxydopamine model. *Mol Ther* 20: 544–554
9. Carty NC et al. (2008) Adeno-associated viral (AAV) serotype 5 vector mediated gene delivery of endothelin-converting enzyme reduces Abeta deposits in APP + PS1 transgenic mice. *Mol Ther* 16: 1580–1586
10. Azzouz M et al. (2004) VEGF delivery with retrogradely transported lentivector prolongs survival in a mouse ALS model. *Nature* 429: 413–417
11. King GD et al. (2008) High-capacity adenovirus vector-mediated anti-glioma gene therapy in the presence of systemic antiadenovirus immunity. *J Virol* 82: 4680–4684
12. Klein RL et al. (2006) Efficient neuronal gene transfer with AAV8 leads to neurotoxic levels of tau or green fluorescent proteins. *Mol Ther* 13: 517–527

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