

Preface

RNA interference (RNAi) refers to the process by which dsRNA molecules silence a target through the specific destruction of their mRNA molecules. Subsequent to the discovery that small interfering RNAs (siRNAs) mimicking the Dicer cleavage products can silence mammalian genes, RNAi has become the experimental tool of choice to suppress gene expression in a wide variety of organisms. In addition, RNAi has also become a method of choice for key steps in the development of therapeutic agents, from target discovery and validation to the analysis of the mechanisms of action of small molecules. To date, several strategies have been devised to trigger the RNAi pathway, each of which is adapted and optimized for different cell systems. Although the technology has several advantages over other methods, the specificity of gene silencing is not absolute and there is a danger of off-target effects and activation of innate immunity. Also, strategic success of therapeutic siRNAs will depend on the development of a delivery vehicle that can target pathogenic cells and from our understanding of the biogenesis of microRNAs (miRNAs). The purpose of this book is to provide readers with the recent advances in siRNA design, expression, delivery, in vivo imaging, and methods to minimize siRNA unwanted effects and use in patients.

To design an effective siRNA, one must consider the base composition of the chosen site and whether the target site will be accessible. Chap. 1 critically reviews the published design guide rules and presents new statistical and clustering design strategies that are useful for selecting effective siRNA sequences. If the chosen target is an RNA virus that can mutate rapidly, one may consider to target conserved site sequences and/or to combine diverse siRNA sequences.

Recent studies indicated that certain siRNA sequences can activate innate immunity resulting in the production of pro-inflammatory cytokines and type I interferons. Moreover, siRNAs can also silence the expression of unrelated genes, a phenomenon known as off-target effects that is mediated largely by limited target sequence complementary to the seed region of the siRNA guide strand. Unfortunately, the current tools for siRNA design (see Chap. 1) cannot eliminate all the potential unwanted effects of siRNAs. Chap. 2 offers valuable and detailed description of how to eliminate siRNA unwanted effects, including the activation of innate immunity and off-target effects. Also, it describes siRNA-based methods for enhancing tumor immunity.

Notably, some of the main challenges in using siRNAs in vivo are the delivery, tissue targeting, and monitoring of siRNA potency in vivo. In vitro, siRNA duplexes have been delivered to target cells mainly by lipid-mediated transfection or via electroporation. However, these methods are not broadly applicable in patients. Approaches to improve in vivo delivery of siRNAs are currently being pursued using nanoparticles, new lipid formulations, and receptor-mediated targeting. Chaps. 3–8 describe new formulations and strategies with promising applications in vitro and in vivo. While Chap. 5 describes the first multimodal nanoparticles to deliver siRNAs, image siRNA

uptake, and monitor gene silencing in tumors, Chap. 6 describes the first detailed protocol for siRNA magnetofection that is applicable in vitro and in vivo.

Being RNA, siRNAs are prone to nuclease-mediated degradation in serum and the cytosol, which has a negative impact on their use in cells and patients. Chemical modifications of ribose (e.g., locked nucleic acids, 2'-deoxy, 2'-fluoro, 2'-O-methyl) can enhance nuclease resistance without interfering with siRNA silencing potency. Chap. 9 describes the development of nuclease-resistant siRNAs with the potential to progress into a new class of therapeutic drugs. Chap. 10 describes new vectors for RNAi in which a synthetic siRNA/miRNA is expressed from a synthetic stem-loop precursor based on the miRNA 155 and miRNA 30 precursors. These new vectors offer several advantages over traditional RNAi vectors driven by RNA polymerase III promoters. These include the expression of several artificial miRNAs from a single transcript and tissue-specific expression as discussed in Chap. 3.

By using siRNAs to downregulate gene expression in human cells, a number of therapeutic target genes have been validated both in vitro and in vivo. Several relevant examples are featured in Chaps. 11–15. These include oncogenes, growth factors, immune regulatory factors, urokinase plasminogen activator and its receptor, matrix metalloproteinases, hypoxia-induced factor, and telomerase.

In addition to interfering with endogenous genes, siRNAs have been used to block viral replication. Nevertheless, in vitro and in vivo experiments have revealed potential problems of viral escape mutants. Chap. 16 describes the treatment of respiratory viral diseases with chemically modified new generation of siRNAs. And Chap. 17 describes the recent progress in using siRNAs as treatment for HIV-1 infection and several excellent recommendations are offered.

Notably, the success of siRNAs will depend not only on the development of delivery strategies and chemical modifications, but also on our understanding of miRNA biogenesis. Naturally occurring miRNA are 19–24 nt in length cleaved from 60- to 110-nt hairpin precursors that are produced from large primary transcripts. To date over 1000 miRNAs have been identified in humans. They play critical roles in developmental and physiological processes by regulating target gene expression at the post-transcriptional level. It is therefore not surprising that deregulation of miRNA expression could result in specific disease phenotypes. Recent studies have implicated miRNAs in cancer development. A group of three chapters describe the recent progress in understanding miRNA expression, function, and involvement in diseases. Chaps. 18 and 20 focuses on the recent progress in understanding the components involved in miRNA function, biogenesis, and interference with virus infection, and Chap. 19 demonstrates that intron-derived miRNA can induce RNAi not only in vitro but also in adult mice. Chap. 21 describes the design of effective miRNA sequences and their applications as anti-gene agents. The book ends by describing the first clinical trial in a patient with leukemia using a synthetic siRNA against *Bcl-Abl* fusion transcript (Chap. 22). It was found that the use of siRNAs in humans is safe, thus facilitating the progression of synthetic siRNA-based drugs to clinical trials.

Topics covered in this volume will be of interest to researchers, teachers, students, and biotech companies interested in RNAi, gene regulation, and gene and immunotherapy. It is my hope that the readers will benefit from this collection of

excellent chapters dealing with the recent advances of RNAi technology from the bench to bedside.

Finally, I would like to thank the authors for their contributions, Anne Dybwad for excellent editorial assistance, the series editors, John Walkers, and all those involved in the production of the book.

Mouldy Sioud

1. Methods for Selecting Effective siRNA Sequences by Using Statistical and Clustering Techniques	1
<i>Seigoro Takataki</i>	
2. Deciphering the Code of Innate Immunity Recognition of siRNAs	41
<i>Mouldy Sioud</i>	
3. Targeted Delivery of Antisense Oligonucleotides and siRNAs into Mammalian Cells	61
<i>Mouldy Sioud</i>	
4. Local and Systemic Delivery of siRNAs for Oligonucleotide Therapy	83
<i>Fumitaka Takehira, Naomi Hokenada, Kimi Homma, Agnieszka Panas, and Takahiro Ochiya</i>	
5. Imaging of siRNA Delivery and Silencing	93
<i>Anna Moore and Zdravka Medarova</i>	
6. Recent Advances in Magnetofection and Its Potential to Deliver siRNAs in Vitro	111
<i>Olga Mykhaylyk, Olivier Zelphari, Edelburga Hammerich, Martina Anson, Joseph Rosenauer, and Christian Plank</i>	
7. In Vitro and In Vivo Gene Silencing by Transkingdom RNAi (tkRNAi)	147
<i>Shuanglin Xiang, Andrew C. Kozlov, Johannes Pruchnik, Yunxin Yang, Hongwen Guo, Thi Nguyen, and Chong J. Li</i>	
8. Bacterial Delivery of siRNAs: A New Approach to Solid Tumor Therapy	161
<i>De-Qi Xu, Liqi Zhang, Dennis J. Kapucko, Lifang Guo, Yuxing Siao, Baofeng Guo, and Lifeng Zhao</i>	
9. The Therapeutic Potential of LNA-Modified siRNAs: Reduction of Off-Target Effects by Chemical Modification of the siRNA Sequence	189
<i>Kees Hutter, Olaf R. F. Mook, and Frank Baas</i>	
10. pSM155 and pSM50 Vectors for miRNA and siRNA Expression	205
<i>Jinshu Wu, Akua N. Bonora, and Guangwei Du</i>	
11. Targeting Oncogenes with siRNAs	221
<i>Olaf Heinemann</i>	
12. Targeting Stromal-Cancer Cell Interactions with siRNAs	243
<i>Neydherlein Afarinejad, Mouldy Sioud, Oran Lucas, and Doreen Abraham</i>	