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## Preface

Subsequent to the discovery that small interfering RNAs (siRNAs) mimicking the Dicer cleavage products can silence mammalian genes, RNA interference (RNAi) has become the experimental tool of choice to suppress gene expression in a wide variety of cells and organisms. The catalytic potency of siRNAs lies in the key discovery that endogenous RNAi gene silencing machinery can be hijacked to artificially knock down genes of interest. Today, 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 types. Although considerable progress has been made recently in understanding how gene silencing is mediated by the RNAi pathway, the rational design of therapeutic siRNAs devoted to off-target effects is still a challenging task. Also strategic success of therapeutic siRNA or micro (mi)RNAs will depend on the development of versatile delivery systems due to the poor cellular uptake of naked RNA molecules. The purpose of this book is to provide readers with the recent advances in siRNA design, delivery, targeting, and methods to minimize siRNA unwanted effects. Preclinical and clinical use of synthetic siRNAs, the roles of miRNAs in cancer, and the promise of extracellular miRNAs for diagnosis are also covered in this issue along with novel methods for identifying endogenous siRNAs and annotation of small RNA transcriptomes.

To design an effective siRNA sequence, one must consider the base composition of the chosen target site and whether it will be accessible. Delivery methods should be also considered when dealing with primary cells, preclinical, and clinical studies. Chapter 1 critically reviews several parameters such as design, delivery, and chemical modifications that are important for successful siRNA applications. Nanostructured RNAs capable of inducing RNAi and new shRNA constructs are described in Chapters 2 and 3, respectively. With respect to delivery, new siRNA formulations, including polymers, PGLA microspheres, magnetic nanoparticles, microwell-based transfection as well as bacteria-based approaches, are adequately described in Chapters 4–8.

The efficacy and safety of siRNA drugs heavily depends on their delivery to the intended target. Selectively targeting siRNAs to diseased cells or tissues increases their accumulation at the site of interest, thus increasing the silencing potency and limiting toxicity to normal tissues. While Chapter 9 deals with direct injection of siRNAs into tumors, Chapter 10 describes a strategy for siRNA delivery to hepatocytes for the treatment of chronic hepatitis B virus infection. Interestingly, the addition of a hepatocyte-targeted endosome-releasing agent enhances gene silencing. Local delivery of siRNAs avoids systemic exposure and reduces the likelihood of unexpected harmful effects elsewhere in the body as results of the unwanted siRNA effects. Chapter 11 deals with the development of aptamers, which are in vitro-evolved RNA or DNA oligomers that bind to target ligands with a high degree of specificity. Chapter 12 critically reviews the currently used targeting strategies for directing siRNA molecules to desired cells, including peptides, antibodies, and CpG oligonucleotides.

Being RNA, siRNAs are prone to nuclease-mediated degradation in biological fluids, which has a negative impact on their use in patients. Chapters 13 and 14 describe the development of nuclease-resistant siRNAs with the potential to progress into a new class of therapeutic drugs against virus-related diseases and cancers.

Notably, the enthusiasm for siRNA-based therapies is reflected by the large number of pharmaceutical companies pursuing this strategy. siRNA-targeted therapies could also be used to enhance or to prevent resistance to standard chemotherapeutic agents or other biological agents. Chapter 15 describes the design of a new vaccine formulation (dendritic cells loaded with tumor antigens that have been transfected with an interleukin-10 siRNA) capable of killing leukemic cells in a rat model of acute myeloid leukemia. Chapters 16 and 17 describe the use of siRNA in cancer patients. Reprogramming DC vaccines with siRNA targeting immunosuppressive factors enhanced DC function and clinical responses in patients with ovarian cancer. Moreover, targeting *bcr-abl* transcripts in a patient with chronic myeloid leukemia with siRNA resulted in inhibition of BCR-ABL, which led to chronic myeloid leukemia cell apoptosis without any associated adverse effects that could be ascribed to the siRNA drug. These clinical studies should facilitate the progression of synthetic siRNA-based drugs to clinical trials.

Although siRNAs are known to induce specific degradation of the target RNA in a sequence-dependent manner, some concern has been raised about the specificity of gene silencing. To overcome this challenge, multiple statistical and computational models have been proposed in recent years to design functional siRNAs. Regardless of the method used, in my opinion unwanted effects mediated by both the sense and antisense siRNA strands cannot be eliminated. Chapters 18 and 19 provide researchers with reliable means to minimize off-targeting concerns associated with RNAi experiments.

Small noncoding RNA molecules such as miRNAs, are abundantly expressed in all cell types and are involved in the regulation of key cellular process such as metabolism, proliferation, DNA repair, apoptosis, and differentiation. Dysregulation of certain miRNAs expression in the cell was consistently observed during certain pathologies including cancers. Chapter 20 critically reviews the miRNA field and describes a method for gene silencing using intronic miRNAs. Chapters 21–23 describe new strategies for in silico identification for novel endogenous siRNAs, annotation of small RNA transcriptomes, and a miRNA capture affinity method, respectively. Chapters 24 and 25 critically review the involvement of miRNAs in cancers and critically discuss the rationale and the strategies for the therapeutic targeting of miRNAs.

Recent studies have shown that, in addition to being expressed endogenously, miRNAs can be secreted from cells. Most of these extracellular miRNAs are associated with lipid carriers known as exosomes, which are membrane-derived vesicles. A significant amount of miRNAs were detected in all biological fluids including blood plasma, urine, and cerebrospinal fluids. The diagnostic potential of extracellular miRNAs for liver injury, cardiovascular, neurological, autoimmune, inflammatory, and metabolic diseases has been documented by several studies. Chapter 26 describes the recent advances in using urinary miRNAs as a new class of noninvasive biomarkers in oncology, nephrology, and cardiology.

Circulating exosome-containing small RNAs have been demonstrated in vitro to be taken up by recipient cells and to alter gene expression through RNAi. Chapter 27 describes an immunomagnetic method for the isolation of exosomes from human cell lines. This improved isolation method should have utility in exosome characterization and clinical applications. Previous work has shown that small RNA such as siRNAs and exosome-derived miRNAs can bind to Toll-like receptors (TLR)-7/8 resulting in the activation of innate

immunity. Chapter 28 describes the design of 2'-modified small RNAs capable of blocking TLR-7/8 signaling and thus functioning as antagonists. Given the involvement of TLRs in inflammatory diseases, the development of TLR antagonists might have clinical applications.

Topics covered in this volume will be of interest to researchers, clinicians, teachers, and biotech companies interested in RNA-based therapies. I would like to thank the authors for their contributions, the series editor John Walker, and all those involved at Springer for the production of the book. It is my hope that you will find the book informative and a valuable addition to your textbook and laboratory bookshelf.

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Challenges and Therapeutic Opportunities

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