RNA INTERFERENCE
Application to Drug Discovery and Challenges to Pharmaceutical Development

Edited by

PAUL H. JOHNSON
PhaseRx, Inc.
Seattle, Washington
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RNA interference (RNAi) is a form of posttranscriptional gene silencing within cells involved in the control of gene expression. The RNAi pathway is found in many eukaryotes including animals and humans. It is initiated by the enzyme Dicer, which cleaves long double-stranded RNA molecules into short fragments of about 20 base pairs. The products of this reaction, small interfering RNA (siRNA) and microRNA (miRNA) molecules, bind to other homologous RNAs (transcripts) and affect their activity (protein expression) via degradation or translational inhibition. RNAi functions in defending cells against parasitic genes—viruses and transposons—and also in directing development as well as gene expression in general. The specific and potent effect of RNAi on gene expression makes it a valuable research tool in both cell culture and animal models to systematically shut down each gene in the cell to help identify the components necessary for a particular function/cellular process. RNAi may also be exploited in humans by introducing synthetic siRNA into cells and suppression (silencing) of disease-causing genes.

Exploitation of the RNAi pathway is a promising approach to treat a variety of diseases. Of great importance is the current view that 80% of the genome is not drugable by small molecules (10%) or biologics (10%), but is potentially drugable by gene silencing technologies. RNAi is seen as a promising way to treat cancer by silencing genes differentially upregulated in tumor cells or genes involved in cell division. Other proposed clinical applications include antiviral therapies to treat infection by herpes simplex virus type 2 and the inhibition of viral gene expression in cancerous cells, knockdown of host
receptors and coreceptors for HIV, the silencing of hepatitis A and hepatitis B genes, silencing of influenza gene expression, and inhibition of measles viral replication. Potential treatments for neurodegenerative diseases include muscular dystrophy and polyglutamine diseases such as Huntington's disease.

A key area of research in the use of RNAi for clinical applications is the development of safe and effective delivery systems that permit targeting to specific cell types and tissues with efficient cell uptake and release into the cytoplasm, the site of action. The goal of this book is to bring together a series of critical review and analysis chapters by leading scientists in the RNAi field that assess the key issues in the development of RNAi-based drugs for clinical applications.

The first section of the book covers the biology of RNAi. Chapter 1 discusses the origins and overview of RNAi. Chapter 2 describes nucleic acids as regulatory molecules including the artificial modulation of gene expression using antisense technology, triplex-forming oligonucleotides, nucleic acid decoys, aptamers, ribozymes and DNAzymes, and RNAi by siRNAs and miRNAs. Chapter 3 examines the use of siRNA oligonucleotides to study gene function including siRNA design strategies, target specificity, chemical modification, delivering siRNA in cell culture, and the experimental design and detection of gene silencing (knockdown). Chapter 4 explains genome scanning by RNAi, dissection of physiological and pathological processes with genetic screens, large-scale RNAi-based screens in mammalian cells, and the design and practical implementation of high-throughput RNAi screens.

The second section of the book deals with the development of siRNA for therapeutic applications. Chapter 5 discusses the discovery of siRNA delivery agents, focusing on issues and challenges related to cellular uptake mechanisms and tight junction dynamics, peptide-based delivery, targeting specific cell types, and high-throughput screening approaches. Chapter 6 describes the potential for use of delivery systems for synthetic siRNAs to overcome the limitations of siRNAs and enhance therapeutic efficacy including current limitations to delivery of siRNAs in vivo, interferon induction and the innate immune system, siRNA administration without delivery assistance, nonviral delivery vehicles for siRNAs, physical delivery, synthetic siRNA delivery systems, pegylation of siRNA delivery vehicles, cell targeting ligand conjugation to siRNA delivery vehicles, and the rational design of modular multicomponent siRNA delivery systems. Chapter 7 focuses on immunologically based in vivo toxicities including the mechanisms of nucleic acid-mediated immune stimulation, Toll-like receptors, TLR-independent mechanisms and protein kinase R, factors influencing immune stimulation, and the implications for pharmaceutical product development. Chapter 8 discusses synthetic siRNA drug development and therapeutic applications for respiratory syncytial virus and influenza viruses and describes the application of RNAi to viral diseases.
focusing on proofs of concept for therapeutic RNAi treatment of virus infection, viral countermeasures for RNAi, translating siRNA delivery to the clinic, and RNAi versus traditional antiviral drugs.

Currently, there are more than 80 gene silencing therapeutics in clinical trials (http://clinicaltrials.gov/). They have the potential to provide a new class of broadly applicable therapies with clinically relevant efficacy and safety. Efficient delivery and cost-effective manufacturing are major challenges that must be met to achieve success. While current manufacturing processes are not yet cost-effective, they are likely to show significant improvement over time. No current delivery system appears to have the desired efficiency, cell selectivity, and safety profile necessary for an RNAi therapeutic to have a clear clinical advantage over other classes of drugs. However, on the basis of recent results and promising new systems under development, there is reason to believe that this will change in the near future.

RNAi therapeutics, a new class of drugs with unprecedented specificity, efficacy and safety, have the potential to revolutionize drug development and the future of medicine by providing broadly applicable therapies against targets that are currently undrugable.

Paul H. Johnson

Seattle, WA
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FOREWORD

RNA interference (RNAi) has rapidly progressed from an intriguing scientific discovery to a powerful tool for studying gene function. In addition to the use of RNAi in the research laboratory, many investigators and commercial ventures are exploiting its potential as a sequence and gene-specific therapeutic agent. The idea that an oligonucleotide could be used to block gene function by virtue of Watson–Crick base pairing is not new, but was described over three decades ago by Paul Zamecnik and colleagues. The original antisense oligonucleotides were largely composed of DNA with various backbone modifications to stabilize the oligos or alter their Watson–Crick binding stability. For various reasons, with a few minor exceptions, the potential of antisense oligos to serve as functional genomic tools or therapeutic agents has never been fully realized. Since RNAi is in effect a form of antisense, why is it so much more potent than the conventional oligonucleotide approaches? Perhaps the biggest difference between RNAi and the antisense DNAs is that RNAi engages a specific set of cellular proteins that have evolved over millions of years for regulating gene expression. In contrast, antisense DNA approaches relied upon diffusion of the oligo to the target mRNA sequence wherein RNaseH is recruited to cleave the RNA. The two phenomena are similar in that the major mammalian Argonaute protein effector of RNAi, Ago2, has an RNaseH-like cleavage domain at the active site. The association of Ago2 with other components of the RNAi machinery provides efficient target recognition and results in target destruction following Ago2-mediated cleavage. Thus, it is fair to state that RNAi is the most powerful sequence