METHODS IN MOLECULAR BIOLOGY™

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Therapeutic Applications of RNAi

Methods and Protocols

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Preface

In the short time since its discovery, RNA interference (RNAi) has become a well-established tool in the drug discovery process. The ability to knock down expression of any gene in mammalian cells using a straightforward method has revolutionized the processes of target identification and validation, and helped to usher in the era of functional genomics. RNAi is a process in which cytoplasmic long double-stranded RNAs (dsRNAs) produced by viral infection, transposons, or introduced transgenes are targeted for inactivation. These long dsRNAs are processed into 21- to 23-nucleotide RNA duplexes by an RNase called Dicer, and are further incorporated into an RNA-induced silencing complex (RISC). The RISC uses these small RNAs to identify and cleave homologous mRNAs in the cell.

RNAi has been used to interrogate the function of candidate genes and, more recently, following the creation of random and directed siRNA libraries, has permitted phenotype-driven, reverse genetic analysis of normal physiological and disease processes. The development of stable and inducible expression vectors driving the expression of short hairpin RNAs has further expanded the application of RNAi both in tissue culture and in animal models.

Beyond its utility as a research tool, the use of RNAi as a therapeutic method promises to bring about an even greater revolution in drug discovery. The key features of RNAi – its high degree of specificity, the ubiquity of its mechanism in all cell types, its catalytic nature, and its ability to target virtually any gene in the genome – have generated excitement that RNAi-based drugs may soon become a reality. While the field of RNAi-based therapeutics is still in its early stages, research is moving ahead rapidly, with several siRNAs already being tested in the clinic.

However, significant hurdles remain, with drug delivery key among them. Delivering siRNA molecules to the right tissue and cell type to treat disease is difficult. Getting siRNAs across the cell membrane and into the cytoplasm where they can effectively silence their targets poses significant challenges. And formulating RNAi-based drugs in ways that allow convenient administration is a difficult problem as well. Currently, many delivery methods are being explored, and many have shown promising results in preclinical models. In some cases, local delivery of an unmodified siRNA may be possible with nothing more than a simple excipient as formulation. But in most cases, some sort of delivery vehicle is required. Many classes of delivery agents are under investigation, including liposomes and lipoplexes, conjugates, polymers and nanoparticles, peptides and proteins. In all of these cases, the challenge is to couple effective delivery with acceptable safety.

With few RNAi programs yet in the clinic, most of the focus remains at earlier stages: testing of delivery vehicles, identifying appropriate model systems, and evaluating the effects of RNAi in vivo. The chapters in this volume address these aspects of research toward the therapeutic application of RNAi. They describe the therapeutic applications of RNAi and potential pitfalls in oncology, viral infections, and CNS disease, using a variety of delivery methods, including liposomes, peptide-based nanoparticles, polycationic polymers, and viral vehicles. In all cases, detailed protocols are provided, to allow

application of these techniques in the reader's laboratory. The collection of essays, by a team of internationally renowned authors, includes basic science chapters dealing with the biology and design of RNAi, essays describing novel strategies for delivery in vivo, and papers that discuss the application of RNAi in a variety of therapeutic areas. This volume will be of interest to basic and clinical researchers, biochemists, clinicians, molecular biologists, physiologists, and pharmacologists.

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