Tech News

siRNA and microRNA

In Control

Small RNA molecules, processed from larger RNA molecules, play roles in the regulation of gene expression by binding to target genes. Small interfering RNAs (siRNAs), generally 21–25 nucleotides in length, bind to target mRNA, probably through perfect (or close) base-pairing, and initiate sequence-specific cleavage. MicroRNAs are small, endogenous RNAs, usually 19–25 nucleotides in length that are cleaved from hairpin structures. Hundreds of microRNAs have been identified, and each is thought to have hundreds of target genes. They regulate these target genes by inhibiting the translation and processing of transcripts, probably through imperfect base-pairing. MicroRNAs are involved in normal development processes, including cellular growth, cell differentiation, and programmed cell death. MicroRNAs also play a role in disease processes, including tumor development, age-related disease, and disorders of the immune system, cardiovascular system, and nervous system.

RNA interference (RNAi)—a mechanism of gene silencing via these small RNAs that was originally described in plants and invertebrates—is known to occur in mammalian cells, and therapeutic applications of RNAi using both siRNA and microRNA are being developed. Some applications are based on the knowledge that RNAi is used in some organisms to defend against viruses and genetic “invaders” (e.g., transposable elements), and that synthetic siRNAs can be active in RNAi in mammalian cells. Among the remaining challenges that need to be addressed are techniques for determining the correct sequence for the therapeutic RNA, delivering that RNA to the appropriate site, and protecting it from rapid degradation.

Topical Protection

Deborah Palliser, Assistant Professor of Microbiology and Immunology at the Albert Einstein College of Medicine in the Bronx, NY, and Judy Lieberman, Professor of Pediatrics at Harvard Medical School in Boston, MA, recently published the results of their collaborative study in *Cell Host & Microbe*. They

Figure 1. Lipid complexes have been used to increase siRNA longevity, but some of these complexes may have toxicity issues. Left (no treatment): CD45+ cells (red) present in the lamina propria (all cells stained blue). Right (oligofectamine): following application of the transfection lipid reagent oligofectamine, there is a small, but significant influx of CD45+ inflammatory cells. Courtesy of Yichao Wu, Albert Einstein College of Medicine.

showed that a topical microbicide preparation silencing two genes via RNAi conferred long-lasting protection against genital herpes caused by herpes simplex virus 2 (HSV-2) in a mouse model. The two genes targeted by siRNA were UL29, a viral gene required for replication; and the gene for nectin-1, a protein on the surface of vaginal cells that is used by HSV-2 to enter those cells. The viral gene suppression is immediate, but short-lived, lasting only ~1–2 days. Though the nectin-1 cell-surface receptor gene is not silenced until several days after application, viral challenge indicated that its suppression gave protection for 1 week. Topical application of a solution containing the therapeutic construct is protective if administered 1 week before to a few hours after exposure to HSV-2. The microbicide does not cause inflammation of vaginal tissue, an effect seen with other siRNAs.

Palliser started this work while studying for her Ph.D. in Lieberman’s laboratory. Now, she is using questions that arose during her time as a post-doc to influence her research at Einstein. She says that toxicity of siRNAs has been an issue, as well as the fact that the molecules are incredibly short-lived. Lipid complexes have been used to increase longevity of siRNA, but some of these complexes, despite their longevity, still have toxicity issues. As shown in Figure 1, application of the transfection lipid reagent oligofectamine resulted in a small, but significant influx of CD45+ inflammatory cells. Palliser says, “This was one of the early findings that prompted us to investigate methods of siRNA delivery that did not require lipid reagents and lead us to using the cholesterol-conjugated siRNAs. The most exciting and significant questions for therapy are [about how] to have as

few side effects as possible with long-lived protection.”

One follow-up project Palliser is pursuing is targeted delivery of siRNA to specific cell types to restrict side effects. One of her interests is dendritic cells, which play a role in the activation of immune responses to microorganisms invading mucosal surfaces. Another issue to investigate is what the long-term effects of downregulation of host cell genes (e.g., nectin-1) will be. Nectin-1 is involved in the formation of tight junctions, so the use of an anti-HSV-2 therapeutic that disrupts tight junctions on a long-term basis might not be good. However, tissues to which this therapy is applied are sloughed off on a cyclical basis, Palliser explains, so side effects may be limited. Possible therapeutic applications include use as a prophylactic to infection or to treat reactivation of the virus in already-infected individuals.

General issues involved in using siRNA include not only toxicity and half-life, but how best to deliver a therapeutic agent and whether to target endogenous or viral genes, or both. The observation that siRNAs are taken up not only by the epithelial layer, but also, surprisingly, by the lamina propria under the cell layer, has generated interest in how general this phenomenon is to other mucosal tissues, how siRNAs traverse the epithelial layer, and what receptors are involved. “We need an in vitro system to screen. It’s difficult to know how to set one up. We’re interested in looking at this and have thought over the last few years how to address it,” she says.

Lieberman agrees that delivery of siRNAs is one of the main obstacles in RNAi therapeutics. She says that it’s also important to make sure that the effect of RNAi-based therapies is indeed due to gene silencing, and not to activation of

the immune system. She will be collaborating with others to develop a topical microbicide that could be used in people. Another area of interest is applying this technology to human immunodeficiency virus (HIV).

Disease Profiles

Sophia Adamia of the Dana-Farber Cancer Institute at Harvard Medical School in Boston, MA, is looking at microRNA expression profiles in patients with multiple myeloma and Waldenström macroglobulinemia, as well as using microRNAs to find novel therapeutic targets for leukemias. This work is based on collaborations among Dr. Nikhil Munshi and Dr. Steven Treon at Dana-Farber, and Dr. Herve Avet-Loiseau at the Laboratoire d'Hématologie Institut de Biologie in Nantes, France. Adamia's group is using quantitative reverse transcriptase polymerase chain reaction (RT qPCR)-based microarray technology to do microRNA profiling on patient samples, in an effort to determine microRNA patterns associated with prognosis and clinical outcome. One obstacle of these types of studies is that any given patient will have different percentages of malignant cells in the bone marrow. There are methods to enrich for the malignant cells, such as using magnetic bead selection technology. Using antibodies to the cell-surface protein marker CD138 attached to magnetic beads for the selection of malignant cells from patients with myeloma is well-established. This method is further validated using the gold-standard method of flow cytometry. For patients with Waldenström macroglobulinemia (WM), CD19-positive B cells can be selected using the same methods. However, other cells should be collected from the bone marrow aspirate of WM patients in order to track down cells truly responsible for the malignancy.

To implement a routine diagnostic microRNA profiling test in the clinic, it is necessary to conduct this test on the blood samples of patients. However, in patients with multiple myeloma, the percentage of circulating malignant cells in the blood is low, so Adamia would like to compare microRNA signatures of those in the blood to those in the bone marrow, as well as to look for stem cell-like progenitors. She has shown that different microRNAs are up- and down-regulated in myeloma, and that there are patterns of expression associated with either indolent or aggressive disease, all with clinically distinct outcomes. She thinks that it will be possible to obtain an accurate

and distinct picture of the disease state if patients can be screened for microRNA profiles. These microRNA profiles will be useful in predicting survival and patient response to therapy. She would also like to be able to see how WM progresses to multiple myeloma in some patients. "At this moment we do not know which WM patients progress to myeloma. It would be ideal if we could predict that," she says.

At the moment, microRNA profiling is very expensive, but Adamia hopes this will soon change. "Since the field of nanotechnology and nanomedicine is advancing rapidly, eventually we will have tools to screen every single patient who comes to the clinic, and then we could move towards optimal individualized therapy," she says. "It's most important to take our findings from the lab bench to bedside at low cost, so these tests will be accessible to everyone who walks into the clinic." She feels that with more sensitive technology, it will also be possible to determine microRNA profiles at the single-cell level to further refine early diagnosis, disease monitoring, drug response, and new drug development. She anticipates the successful use of microRNAs as therapeutic agents, believing that using these naturally-occurring molecules will enable researchers to balance a system that is out of balance in disease.

Another Application

Scientists at the New York University School of Medicine and the Langone Medical Center in New York, New York, have identified a microRNA that is over-expressed in metastatic melanoma tissues and cell lines. Eva Hernandez, Assistant Professor in the Department of Pathology, explains that increased expression of this microRNA confers oncogene-like activity, increasing the invasiveness of melanoma cells in vitro and in a mouse model. It is associated with a region often amplified in melanoma that contains two other oncogenes, *B-raf* and *c-met*. This microRNA, miRNA 182, is being investigated as a possible therapeutic target to either inhibit the growth or metastasis of primary melanoma, again in a mouse model. Results will indicate whether this will be an approach applicable to human disease.

Written by Lynne Lederman, Ph.D.

BioTechniques 46:257-259 (April 2009)
doi 10.2144/000113112

BioTechniques E-Alerts

BioTechniques Weekly

Weekly newsletter with industry insight for life scientists: news, new products, grants deadlines, employment opportunities, and events.

Advance Online Publication (AOP)

Monthly alert with select research papers published online ahead of the print.

Table of Contents (TOC)

Monthly alert listing current papers and their links allowing you to browse the latest issue immediately.

Register today at

www.BioTechniques.com/newsletters