Targeting Therapeutic Oligonucleotides

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Oligonucleotides are increasingly recognized as potential therapeutic agents for a variety of diseases. This is particularly true for the treatment of genetic diseases and cancer, for which an increasing number of molecular targets are being identified. Moreover, oligonucleotides can — at least in theory — be designed with the use of Watson–Crick base-pairing rules to act on almost any target that otherwise would be “undruggable” by small-molecule entities or by monoclonal antibodies. Recent work1,2 indicates that the usefulness of the technology can be increased by conjugating oligonucleotides with targeting moieties so that they home to specific cell types.

Two major types of oligonucleotide drugs are currently being developed as therapeutic platforms for the reduction of target-gene expression: antisense oligonucleotides and short interfering RNAs (siRNAs). Although these platforms have distinct molecular mechanisms (Fig. 1), they share a fundamental principle: an oligonucleotide binds a target RNA through Watson–Crick base pairing, and the resulting duplex directs degradation of the target messenger RNA (mRNA). Other classes of oligonucleotide therapeutics modulate RNA function by binding to splice sites on pre-mRNAs, which results — for example — in the skipping of mutation-containing exons in diseases such as muscular dystrophy.

An attractive feature of oligonucleotide therapeutics is that they represent a direct application of genomic information in the design of drugs. The concept is straightforward: a target RNA is selected, a complementary oligonucleotide sequence is synthesized, gene expression is modulated, and phenotypes are examined. Reality, however, is more complex. In particular, the inability to deliver the oligonucleotide drug candidate to the organs and cells that are expressing the disease-related RNAs has proved to be the greatest obstacle to the successful development of oligonucleotide therapeutics.

As polyanionic macromolecules, oligonucleotides must clear many hurdles to reach their intracellular site of action. These must be considered in the development of an effective delivery system. Oligonucleotides are considerably larger than traditional small-molecule drug candidates. Their size, coupled with their highly anionic nature, makes it difficult for them to diffuse across cell membranes to reach the cytoplasmic and nuclear compartments.

Historically, researchers studying oligonucleotides have used different strategies for the delivery of single-stranded antisense oligonucleotides and double-stranded siRNAs. Antisense oligonucleotides administered in saline solution are taken up by cells in the liver, kidney, and parts of the reticuloendothelial system and, to a lesser extent, by other cell types; this occurs through endocytosis or nonspecific binding to cell-surface proteins and movement of the oligonucleotides down concentration gradients into the cytoplasm. Double-stranded siRNAs have been administered in lipid or polymeric nanoparticles,3,4 which results in a large portion of the siRNAs accumulating in the reticuloendothelial system, including in the liver and spleen. Both delivery strategies depend on random binding to cell surfaces or extravasation from fenestrated capillaries in the liver. Although capable of driving dose-dependent pharmacologic effects, the strategies are inefficient, leading to renal excretion of the drug or delivery of the majority of the drug to cells and tissues that are not of therapeutic interest.
Figure 1. Receptor-Mediated Uptake of Therapeutic Oligonucleotides.

Panel A shows targeted delivery of oligonucleotide therapeutics to hepatocytes through asialoglycoprotein receptor (ASPGR)–mediated uptake, as described by Viney and coworkers. The ASPGR binds and internalizes triantennary N-acetylgalactosamine (GalNAc) conjugated with a therapeutic antisense oligonucleotide or small interfering RNA (siRNA) (not shown), which are internalized and then released inside the cell, where they can hybridize to their cognate pre–messenger RNA (mRNA) and induce cleavage of the RNA–DNA heteroduplex. Panel B shows the targeted delivery of oligonucleotides to specific cell types through cell-surface proteins, as described by Sugo and coworkers. A specific cell-surface protein (CD71) binds and internalizes the Fab-conjugated siRNA directed against a disease-related gene. Only cells expressing that specific cell-surface protein will internalize the siRNA. After release of the siRNA from the endosomal or lysosomal compartment, the now-cytoplasmic siRNA can load into the RNA-induced silencing complex (RISC). The loaded RISC can then scan all expressed RNAs for sequence complementarity. When a complementary sequence is detected by hybridization, an enzyme that is part of the RISC cleaves the targeted mRNA, thereby reducing the expression of the disease-related protein. RNAi denotes RNA interference.
More recently, the strategy for delivering oligonucleotide therapeutics has changed from non-specific uptake to targeted delivery. This new approach exploits the potential for certain extracellular receptors to promote cellular internalization of receptor-bound ligands. As explained by Khvorova in this issue of the Journal, therapeutic oligonucleotides are conjugated to a triantennary N-acetylgalactosamine (GalNAc) moiety, which is recognized by a high-capacity asialoglycoprotein receptor expressed on hepatocytes. GalNAc-conjugated oligonucleotide therapeutic agents bound to asialoglycoprotein receptors are efficiently internalized into hepatocytes, and once in the cytoplasm (in the case of siRNAs) or nucleus (in the case of antisense oligonucleotides), the oligonucleotides can modulate the expression of the cognate RNA (Fig. 1A). The GalNAc-mediated conjugate strategy is currently being evaluated in multiple clinical trials, such as the one reported in this issue of the Journal, involving an siRNA–GalNAc conjugate, in which the siRNA portion targets the PCSK9 enzyme. This enzyme binds and degrades the low-density lipoprotein receptor (when bound to low-density lipoprotein) and is a target in the treatment of cardiovascular disease.

The advantages of the targeted delivery strategy are also demonstrated by comparing the results of a pair of phase 2 studies in which the activity of two antisense oligonucleotides against apolipoprotein(a), which is expressed in the liver, were compared. One of the antisense oligonucleotides was nontargeted, and the second was targeted to hepatocytes with the use of triantennary GalNAc. On the basis of the reductions in the mean change in circulating levels of apolipoprotein(a) according to dose, targeted delivery was determined to result in a median effective dose that was one thirtieth of that associated with nontargeted delivery, which clearly underscored the potential advantage of the approach.1

Although considerable progress has been made in targeting hepatic tissue with GalNAc conjugates, there remains a need to target nonhepatic cells specifically and efficiently: a means to achieve this goal could lie in the exploitation of the natural heterogeneity of cell surface receptors. Recently, Sugo and coworkers2 used an siRNA conjugated to a Fab fragment of an antibody to CD71 (transferrin receptor) to produce oligonucleotide-mediated reductions in gene expression in skeletal muscle and heart (Fig. 1B). Taking advantage of the fact that CD71 expression in muscle cells is 5 times that in liver cells, the authors were able to reduce gene expression in skeletal muscle and heart selectively, with no changes in gene expression in liver and spleen.

Ultimately, the success of targeted delivery approaches will depend on the rates of internalization and the specific trafficking of the receptor-bound conjugate within cells. However, the existing data indicate that sufficient quantities of oligonucleotide drug can be internalized to produce meaningful reductions in gene expression. These results, taken together with the progress from the ongoing clinical studies with GalNAc-conjugated oligonucleotides, show that oligonucleotide-based drugs are improving with targeted delivery.

Disclosure forms provided by the author are available with the full text of this article at NEJM.org.

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