The Concept and Application of Antisense Oligonucleotides

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Since the identification of the double-stranded DNA helix by Watson and Crick in 1953, the knowledge of nucleotide structure and function has been an important potential tool in the study and therapy of disease. There is recent clinical evidence that antisense oligonucleotides may be important therapeutic compounds in the clinical therapy of a range of diseases, including infection (viruses and bacteria), oncology, and inflammation. Our laboratory-based understanding of antisense oligonucleotide activity has provided a foundation for their use in several human diseases. Potentially relevant applications include inflammatory bowel disease therapy, psoriasis, transplantation, rheumatoid arthritis, cytomegalovirus retinitis, hepatitis C, and solid tumor therapy. Here we will outline these applications as well as our ongoing clinical trials for Crohn’s disease. [Key words: Antisense; ICAM-1; Crohn’s disease; Alicaforsen®]


Antisense oligonucleotides are used to hybridize to a specific RNA molecule in vivo and thereby to inhibit its subsequent availability. In clinical terms, this is akin to “gumming up a disease-causing protein before it is produced” rather than after it is formed by the body (Fig. 1). This is done by synthesizing specific antisense molecules that bind to the message RNA carrying the instructions to form a disease-causing protein. For antisense molecules, the target RNA is a messenger RNA (mRNA), which subsequently cannot be translated into a protein. Recently, we have developed the ability to develop antisense molecules to many disease-associated mRNA targets using automated technology originating from our knowledge of genomics. Really, all that is required is knowledge of the target gene’s nucleotide sequence so that an antisense oligonucleotide with the complementary sequence can be synthesized. In this way the expression of one gene can be blocked, and the consequent observed effects help to elucidate the physiologic role of the gene and its product.

Selection of the sites in an RNA molecule at which optimal antisense activity may be induced is complex, dependent on the terminating mechanism and influenced by the chemical class of the oligonucleotide. Importantly, each RNA displays a unique pattern of sites of sensitivity. For this reason, studies have shown that antisense activity can vary from undetectable to 100 percent by shifting one of its sequence oligonucleotides by just a few bases in the RNA target.1-2

ANTISENSE TARGET DEVELOPMENT

The explosion of information deriving from the sequencing of the human and other genomes has resulted in the need to create tools to help determine the functions of gene products and the relationships between gene products and to determine which gene products are attractive targets for drug discovery programs. Screening for target antisense inhibitors is ex-
Steps in Leukocyte Emigration from Blood Vessels to Sites of Inflammation in Tissues

Figure 1. Inflammatory cell migration through vascular endothelium is mediated by LFA-1/ICAM-1 binding.

Extraordinarily specific, and the technique has been automated so that targets can be screened and tested quickly. This technology can not only rapidly and efficiently define gene function but it can also validate genes as good therapeutic targets. From research collected from the Human Genome Project and additional research, it is now apparent that each human gene on average results in three to four different proteins via a process called "alternative splicing." The ability to use antisense technology to understand this process and the roles of each alternative splice form is another important attribute of the technology.

Therapeutic Utility

Conceptually, in addition to being an excellent tool for functional genomics (that is, the study of what genes can do and produce), antisense technology may prove to be a revolutionary drug discovery technology. In fact, although much remains to be done, based on more than a decade of progress, it now seems that antisense technology will have even broader therapeutic applications than anticipated.

At least two decades of preclinical work on improving the clinical efficacy of antisense molecules has resulted in several generations of these compounds and improved bioavailability. Early antisense compounds (which are essentially modified nucleotides) were designed to provide stability for these drugs against the numerous enzymes present in the body that otherwise rapidly degrade them. The early generation compounds are given intravenously, typically every two days. Unlike antibodies, these molecules can be given in longer-lasting (second generation molecules) as well as oral formulations. As well, topical administration (e.g., for psoriasis), aerosol administration (e.g., for asthma), and subcutaneous administration of newer-generation molecules are in development. These advancements are very exciting given the enormous range of targets made available by this technology. Several antisense compounds have now been evaluated for safety in animals and more than 2,000 patients. One drug, Vitravene®, (ISIS Pharmaceuticals, Carlsbad, CA; an antisense molecule targeting cytomegalovirus retinitis and given intracellularly) has in fact been approved by regulatory agencies around the world. This body of work has demonstrated that members of this chemical class are well tolerated.

Antisense to ICAM-1 for Crohn's Disease

As an early application of antisense drugs in clinical practice, intercellular adhesion molecule (ICAM)-1 was chosen as a potential target for antisense therapy in human disease because it serves an important role in the persistence of an inflammatory response by several mechanisms. ICAM-1 is a member of a group of molecules called "adhesion molecules." Primarily, ICAM-1 is associated with the migration and adhesion of leukocytes from the intravascular space into tissue in response to inflammatory stimuli. Lymphocytes and polymorphonuclear leukocytes expressing ICAM-1 circulate with the blood. When these cells reach an area of inflamed tissue expressing its receptor, lymphocyte function associated antigen (LFA)-1, the cells stop circulating, bind and migrate through the vessel endothelium to participate in the normal or pathologic inflammation in that tissue. Normal gut tissue does not express ICAM-1 in significant levels. As well, the loss of ICAM-1 is not fatal, because the

Figure 2. Antisense technology: the antisense binds to mRNA and activates RNAase H which destroys the complex or alternatively binds and inhibits the translation. (Reproduced with permission, Adis press, 2001.)